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PLANT PHYSIOLOGY IN THE U.S.S.R.

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The extraordinary richness and variety of plant life on an enormous territory, embracing almost one-sixth of the land surface of the world, naturally impelled the Russian botanists first to direct their efforts to the study of the specific composition of its flora and of the geographic distribution of the plants. Classification and phytogeography were therefore the first branches of botany to be developed and still remain the predominant botanical disciplines in the U.S.S.R., both in the number of scientific specialists and in the amount of scientific research work accomplished.

Nevertheless, the beginning of plant physiology refers to the sixties of the last century, the founder of the first physiological school being A. S. FAMINTSYN (1835–1918), Professor of the St. Petersburg University and member of the St. Petersburg Academy of Sciences (since 1889). He published the first university text-book on plant physiology (1887) and a large monograph *Metabolism and transformation of energy in plants* (1883). His first pupils were BARANETZKY and BORODIN.

Plant physiology began to develop towards the end of the 19th and in the beginning of the 20th century, and there appeared consecutively the schools of C. A. TIMIRIASEV, V. I. PALLADIN, S. P. KOSTYCHEV, D. N. PRIANISHNIKOV, A. A. RICHTER, V. V. K. ZALESSKY, V. N. LUBIMENKO, N. A. MAXIMOV, N. G. KHOLODNY.

There was, however, an especially marked rise in the development of plant physiology after the October Revolution, which gave a great impetus to the organisation of new universities, high schools, botanical gardens, and large agricultural experimental institutions, wherein laboratories of plant physiology began to appear. Cadres of young physiologists of the new generation began to grow rapidly. In the Soviet laboratories they receive a complete special education. A great change is also to be observed in the general tendencies of research work. The old direction in plant physiology, as reflected in large handbooks and in text-books, in essence gave only a physico-chemical analysis of separate, unconnected functions of the organ-

ism; the organism as a living, dynamic, whole system was completely absent from them.

At the present time the attention of experimental workers is being more and more centered on the organism itself and on its proper internal factors, controlling all the physico-chemical reactions occurring within it. Consequently not only *phylogenetic variability* (manifesting itself in different species, races, and varieties, in connection with ecological adaptation to environment) is now taken into account even in ordinary biochemical investigations, but likewise *individual variability*, characterizing different stages of ontogenetic development.

The influence of each separate external factor on one or another reaction in the organism is not now studied by means of its simple isolation, as was the method formerly, but on the background of different combinations of other factors. As a result there has been produced an essential modification in our notions of the cardinal points, minimum, optimum, and maximum in physiological functions. They have ceased to be immovable points of a single curve; they have become dynamic, as a reflection of the dynamism of the organism itself, of the change in state of the protoplasm. The earlier investigations on photosynthesis (LUBIMENKO, KOSTYCHEV, L. A. IVANOV, and their collaborators, CHESNOKOV and BAZYRINA) have now given along this new line of investigation and study most interesting results in the works of A. C. DANILOV (the influence of light of different spectral composition), V. A. BRILLIANT (the influence of the water content of leaf tissue), A. K. TOSHCHEVIKOVA and A. L. KURSANOV (the influence of the accumulation of assimilants in the leaf).

The same line of research is followed in the studies on frost resistance, heat resistance, drought resistance (N. A. MAXIMOV, I. I. TUMANOV, A. A. RICHTER, V. N. LUBIMENKO, and collaborators), on mineral nutrition (O. A. WALTER, D. A. SABININ and their collaborators), and on nitrogen nutrition (D. N. PRIANISHNIKOV and collaborators).

The problems of growth and growth movements have shown, after the old works of PORODKO, NELJUBOV, and ROTHERT, an exceptionally brilliant development in the works of N. G. KHOLODNY, who has together with WENT created the hormone theory of growth.

During the last ten years a special and most important place has been won by works on the physiology of development of the higher plants. GARNER and ALLARD'S discovery of photoperiodism, and LYSENKO'S discovery of the temperature yarovisation of seeds have served as a powerful impulse in directing research work along this path.

The favorable results in agricultural practice of the method elaborated by LYSENKO of the preliminary yarovisation of seeds have led to a wide development, in the greater part of the physiological laboratories of our

Union, of research work on the physiology of growth and development of plants. Attempts are now already being made of synthesizing all of the accumulated data into a theory of development (the most important theory of development-stages of LYSENKO, the hormone theory of LUBIMENKO, KHOLODNY, CHAILAKHIAN). Data have also been obtained for the practical application of photoperiodism in plant culture (the investigations of the laboratories of LUBIMENKO and MALCHEVSKI). An essential feature of the physiological research work of the present day is the choice of the problems studied. They all concern questions having the greatest practical significance for the agriculture and industry of the U.S.S.R. The necessity of such a choice of problems is the natural result of the necessity of replacing the empirics which formerly prevailed in industrial practice by methods scientifically elaborated and approved.

It is of course self evident that such an organization of work does not in the least exclude investigations of the most profound theoretical questions, which are studied in the laboratories of our academies and universities.

At the same time the socialistic structure of the whole economy of the U.S.S.R. guarantees a very speedy penetration into practice of valuable scientific attainments and discoveries. The following may be cited as an example: LYSENKO began to apply his method of preliminary yarovisation of seeds to field cultures in 1931, and already millions of hectares are being sown with yarovized seeds.

In conclusion I wish to emphasise that the high estimation of scientific research work, both by the Government and by society, create in the U.S.S.R. most favorable economic and moral conditions for scientific work and fully guarantee the development of science in general, and of plant physiology in particular.

BOTANICAL GARDEN
LENINGRAD, U.S.S.R.

ASSIMILATION OF AMMONIUM AND NITRATE NITROGEN FROM SOLUTION CULTURES BY ROOTS OF *PANDANUS VEITCHII* HORT., AND DISTRIBUTION OF THE VARIOUS NITROGEN FRACTIONS AND SUGARS IN THE STELE AND CORTEX[1]

C. P. Sideris, B. H. Krauss, and H. Y. Young[2]

(WITH EIGHT FIGURES)

Introduction

In the course of studies on the assimilation of ammonium and nitrate nitrogen by pineapple roots, to be presented in a future publication, it became necessary to follow the conversion of inorganic to organic nitrogen in small lineal regions of the roots and also in the cortex and stele of these regions. As the diameter of pineapple roots is very small and a separation of the tissues of the stele from those of the cortex presents great difficulties, roots of *Pandanus veitchii* were employed. Shoots from plants grown on the campus of the University of Hawaii were placed in water cultures containing respectively ammonium and nitrate salts as sources of nitrogen.

The conversion of inorganic to organic nitrogen was followed in cortex and stele of different regions of the root either by analyzing such tissues or by analysis of the exudate which was collected in flasks under aseptic conditions from the excised end of a certain number of roots.

Literature review

This being the first of a series of papers to follow on the assimilation of ammonium and nitrate nitrogen by different plant tissues, the literature is reviewed rather broadly from the point of view of the entire subject rather than from that of the paper under immediate consideration.

The literature on absorption and assimilation of ammonium salts as sources of nitrogen by different higher green plants has been presented by Parde (**48**) and in a more general manner by Molliard (**36**). However, for a better understanding of the rôle of ammonium nitrogen in absorption, assimilation, and translocation the reader is referred to certain original sources of information. Prianischnikow (**54, 55, 56, 57**) and Prianischnikow and Iwanowa (**58, 59**), who for the last 25 years have studied the various phases of absorption and assimilation of NH_4^+ and NO_3^- at different stages of plant growth, conditions of carbohydrate reserves, pH values, and

[1] Presented at the June, 1935, meeting of the American Society of Plant Physiologists at St. Paul, Minn.

[2] The first of a series. Published with the approval of the Director as Technical Paper no. 95 of the Pineapple Experiment Station, University of Hawaii.

calcium concentrations, found: (1) that NH_4^+ is absorbed at comparatively greater rates at higher pH values than NO_3^- and *vice versa*; (2) that the toxicity characteristic of plants grown in NH_4^+ containing cultures at low pH values is due to H^+ ions and not to NH_4^+; and (3) that NO_3^- ions are reduced first, in conformity with WARBURG's theory (**80**), to ammonia and then converted to amino acids or products of the type of $R(NH_2)(CONH_2)$ (asparagine, glutamine). PRIANISCHNIKOW's findings have been verified and further extended by others with respect to nitrate reduction.

ECKERSON (**14**) has followed the assimilation of NO_3^- ions in different tissues of plants and her findings indicate a reduction of NO_3^- to NO_2^- and a further one to NH_4^+, which results are in harmony with PRIANISCHNIKOW's theory.

The process of NO_3^- reduction is not limited to few tissues or organs and may be found to operate in the roots, leaves, and possibly in the stem according to THOMAS (**69**), NIGHTINGALE and SCHERMERHORN (**45**), NIGHTINGALE and ROBBINS (**44**), DAVIDSON and SHIVE (**12**), TIEDJENS and BLAKE (**72**), HOLLEY, DULIN, and PICKETT (**20**), MASKELL and MASON (**30**), WOO (**81**), LEONARD (**25**), and others.

The relative amounts of NH_4^+ and NO_3^- nitrogen absorbed from solution cultures at high and low pH values have been studied by TSUNG-LE LOO (**77**), EGGLETON (**16**), LEWIS (**26**), TIEDJENS (**72**), TIEDJENS and BLAKE (**73**), CLARK (**8**), CLARK and SHIVE (**9**), DAVIDSON and SHIVE (**12**), STAHL and SHIVE (**64, 65**), PIRSCHLE (**50, 51, 52**), NAFTEL (**39**), MEVIUS (**33**), MEVIUS and ENGEL (**34**), DIKUSSAR (**13**), NIGHTINGALE (**41**), PRIANISCHNIKOW (**55, 57**), etc. Their results indicate that NH_4^+ ions are absorbed and assimilated at considerably greater rates than NO_3^- and that high pH values favor more the absorption of the former and low pH values that of the latter ions.

The effects of different nutrient elements, temperature, light, and other climatic conditions on the absorption and assimilation of NH_4^+ and NO_3^- ions have been studied neither extensively nor very satisfactorily. From the available literature (**6, 17, 18, 19, 22, 28, 30, 39, 41, 42, 43, 47, 49, 62, 71, 74, 75**) we learn that low temperature, small amounts of light, and exceedingly low concentrations of certain nutrient elements retard absorption and assimilation of nitrogen and other elements, and synthesis of proteins.

Materials and methods

Pandanus veitchii shoots weighing between 1 and 4 kilos and having from 2 to 8 aerial roots were employed for these studies. The roots, already formed on the shoots and varying in length from 10 to 50 cm., and in diameter from 0.5 to 2.5 cm., had not yet come in contact with soil nor had they produced any laterals, and from a functional point of view they were virgin as they had never absorbed mineral substances from an external medium.

The tip and distal end, 10 to 15 cm. of each root, were submerged in tap water contained in individual 2-quart Mason jars until an extensive lateral root system was produced. The roots of all different containers were aerated. The time required for the production of an extensive lateral root system was about eight months. The lateral root systems of the different roots were classified into as uniform groups as possible and they were then subjected to various treatments. Certain of such roots were used exclusively for the study of the chemical composition of their exudates and others for that of their tissues. The roots of both groups were divided into smaller lots for different nutritional treatments. These included two types of complete nutrient solutions containing nitrogen from two different sources, that is, either ammonium or nitrate and a third solution which contained no nitrogen but that was otherwise complete. The chemical composition of these solutions is reported in table I.

TABLE I

CHEMICAL COMPOSITION OF NUTRIENT SOLUTIONS

SALT	AMMONIUM	NITRATE	MINUS-NITROGEN
	MOL. CONC.	MOL. CONC.	MOL. CONC.
$(NH_4)_2SO_4$	0.0010	.....	.....
$(NH_4)_2CO_3$	0.0010	.....	.....
KH_2PO_4	0.0005	0.0005	0.0005
K_2SO_4	0.0010	0.0010	0.0010
$MgSO_4$	0.0010	0.0010	0.0010
$CaCl_2$	0.0020	.....	0.0020
$Ca(NO_3)_2$	.....	0.0020	.....
$FeSO_4$*	0.0001	0.0001	0.0001
pH (about)	6.8	5.6	5.4

* A stock solution of $FeSO_4 \cdot 7H_2O$ was prepared by dissolving 346 gm. of the salt in one liter of 0.1/N H_2SO_4. It was kept in a dark bottle and used in the dilution indicated above.

The roots of the plants in the exudate experiments were severed from the stem at the time they were placed in the different nutrient solutions. The cut-off end of the root was led, in all cases, into the mouth of a 125-cc. Erlenmeyer flask, containing 1 cc. of toluene, where it discharged the exudate which was removed from the flask at 24-hour intervals. In cases in which roots were allowed to discharge for more than 48 hours, a portion of the cut-off end, about 0.5 to 1 cm. long, was removed to secure an unobstructed flow and prevent contamination of the exudate with the by-products of dying tissues. The roots of the group for studies of the chemical composition of the tissues included small lateral roots and the main root fractions

which were obtained by crosswise cutting into three parts, (1) the terminal, (2) the intermediate, and (3) the proximal fraction of the main roots. With the exception of the small lateral roots, the tissues of the stele were separated from those of the cortex. All the tissues were weighed before they were placed in individual containers and were analyzed separately. Three different samples were prepared for chemical analysis of each group of tissues: (1) for ammonia and organic nitrogen analysis; (2) for nitrate and sugars; and (3) for drying. In the sample for sugar analyses a small volume of $(Na)_2CO_3$ was added to prevent hydrolysis.

The exudates were analyzed as rapidly as the volume necessary for analysis was collected. The tissues of the roots harvested for analysis were cut into small pieces and placed for 30 seconds in flasks containing a small volume of boiling water (for inactivation of enzymes). The containers were immediately cooled and 5 cc. of toluene added. All samples were analyzed within a period of 2 to 4 weeks.

Preparation of samples

The plant tissues were next strained through small pieces of cheesecloth. The collected liquid was returned to the flask while the solids were placed in a brass mortar and ground with quartz sand. The ground solids in a fine pulpy state were returned to the original flask containing the liquid. Methods generally employed in biochemical analyses with recent modifications (**29**) and improvements have been adopted for ascertaining the chemical composition of the exudates and tissues.

Analyses for ammonia and organic nitrogen

Ammonia.—The entire volume of the ground tissues and liquid was placed in pyrex cylinders, 35.5 cm. long and 4.45 cm. in diameter, to which N/1 NaOH was added to bring to pH 8 and then 5 to 10 cc. depending on the quantities of tissues, of a phosphate-borate buffer of pH 8 (**61**) was added. The battery of 12 cylinders was placed in a temperature bath, maintained at 45° C. The contents of the cylinders were aspirated at 45° C. for 1 hour for small and for 3 hours for larger quantities. The ammonia of the tissues was collected in N/50 H_2SO_4 and was determined by *Nesslerization* or by titration, depending on the amounts present.

Glutamine.—The ammonia-free residue was brought with H_3PO_4 to pH 5 to 6. It was then boiled for 2 hours, evaporated to a smaller volume, cooled, brought with N/1 NaOH to pH 8 and aspirated at 70° C. The ammonia was then determined as already described.

Separation of soluble from insoluble organic nitrogen

The glutamine-free residue was cooled, acidified with acetic acid to pH 3, and toluene was added. It was allowed to stand in the refrigerator over-

night or at room temperature and then filtered. The residue on the filter paper (no. 42 Whatman) was repeatedly washed with boiling water containing 0.5 per cent. acetic acid. The filtrate was very clear and apparently free from suspensions of proteinoid substances. The residue, containing the proteins of the tissues, was placed in a flask together with a definite volume of 20 per cent. HCl and stoppered for later analysis. The filtrate containing different fractions of soluble organic nitrogen was then analyzed according to the following methods.

Analysis of soluble nitrogen fractions

Asparagine.—To the total volume of the filtrate, H_2SO_4 was added to make a 4 per cent. concentration. The mixture was heated, under a reflux condenser, for two hours, and sufficient NaOH was added to make it distinctly but not excessively alkaline (pH 8 to 9). The ammonia formed from asparagine was aspirated at 70° C. as in the method for ammonia. The amounts of amide nitrogen thus obtained were multiplied by 2 to include the amino nitrogen of asparagine. However, the amino nitrogen value was subtracted from the alpha-amino of mono-amino determinations.

Alpha-amino nitrogen.—About 20 cc. of the asparagine-free residue was adjusted with H_2SO_4 to pH 6 and was then evaporated on a water bath to a volume of 4 cc. Two cc. of the residue were employed for the determination of alpha-amino nitrogen, using the method of Van Slyke (**78**). The modified reaction vessel of Koch (**24**) was employed and both temperature and barometric pressure were recorded with each determination for calculating the weight of N_2 gas.

Mono-amino nitrogen.—In certain determinations instead of alpha-amino nitrogen mono-amino nitrogen is reported. This fraction was recovered in the filtrate from the residue of the asparagine determination after treatment with phosphotungstic acid. It is essentially composed in its major portion of mono-amino-carboxylic acids and mono-amino-dicarboxylic acids, and it was obtained as follows: The asparagine-free residue was acidified with 30 cc. of concentrated H_2SO_4, diluted to 300 cc. and then treated with 10 cc. of 50 per cent. phosphotungstic acid depending on the volume of the residue and amounts of basic nitrogen contained therein. The mixture kept at about 4° C. for 40 hours was filtered through no. 42 Whatman filter paper applied to a Buchner funnel, which was fitted through a hole made in a can of greater diameter than the funnel and containing ice. The precipitate was washed repeatedly with acidified phosphotungstic acid at about 5° C.

The filtrate was placed in a Kjeldahl flask where it was digested by adding 5 gm. of anhydrous sodium sulphate and 5 drops of selinium oxychloride. After cooling and the addition of 40 per cent. NaOH, the generated ammonia was distilled in 0.05 N H_2SO_4.

Basic nitrogen.—This fraction representing the quantities of nitrogen contained in such amino acids as arginine, histidine, lysine, and cystine is included in the phosphotungstic precipitate obtained during the separation of mono-amino nitrogen. The precipitate contains, possibly, small amounts of certain other nitrogenous fractions. The precipitate and filter paper were placed in a Kjeldahl flask where they were digested with a mixture of 25 cc. of concentrated sulphuric acid and otherwise treated as already indicated.

REST NITROGEN.—In cases where the extract containing the soluble organic nitrogen was not hydrolyzed beyond the stage of the asparagine determination the residue was employed directly for estimating alpha-amino and rest nitrogen. The alpha-amino was determined by the VAN SLYKE method as already stated. For the estimation of rest nitrogen the previously described Kjeldahl technique was followed on an aliquot of the residue. From the total value of the nitrogen thus obtained that of alpha-amino minus one-half of the asparagine nitrogen was subtracted. The difference represents rest nitrogen which has been possibly derived from some basic nitrogen and from other sources not well known.

ANALYSIS OF INSOLUBLE ORGANIC NITROGEN

The residue or insoluble portion of the tissues, obtained after the removal of glutamine nitrogen, was refluxed, as stated under the heading "separation of soluble from insoluble fractions of organic nitrogen," with 20 per cent. HCl for 24 hours. The hydrolysate was neutralized and filtered. The insoluble residue and filtrate were transferred to separate Kjeldahl flasks, the former containing the humin or melanin nitrogen and the latter different fractions of hydrolyzed protein.

HUMIN NITROGEN.—The total nitrogen content of the flask composed of the acid resistant particles of the tissues was determined by the Kjeldahl procedure already described.

AMIDE NITROGEN.—The filtrate or hydrolysate obtained after the digestion of the dissolved portions of the tissues with 20 per cent. HCl was made slightly alkaline with NaOH, then aspirated, and the ammonium estimated as in the glutamine determination.

MONO-AMINO AND BASIC NITROGEN.—The amide-free residue was neutralized, acidified with 20 cc. of concentrated H_2SO_4, treated with 10 cc. of 50 per cent. phosphotungstic acid, and kept at 4° C. for 40 hours. The determination of the mono-amino nitrogen and basic nitrogen was then made in the same way as already described.

ANALYSES FOR NITRATE NITROGEN AND SUGARS

NITRATE NITROGEN.—This fraction was determined on an aliquot of the sample employed for sugar analysis, using the phenol disulphonic method (**2**).

This method was compared with that of PUCHER, LEAVENWORTH, and VICKERY (**60**) and that of TRESCHOW and GABRIELSEN (**76**) and was found very satisfactory for the plant material concerned. The tissues were extracted repeatedly with water until the final weight was 10 times greater than the initial weight of the tissues. An aliquot of the extract was cleared with lead acetate and the filtrate from the precipitation was deleaded with Na_2HPO_4, and filtered. An aliquot of the filtrate was evaporated over a water bath to dryness, then treated with phenoldisulphonic acid, water, and ammonium hydroxide (**2**). With samples containing certain pigments interfering with the accuracy of the colorimetric determination a small amount of norite was added. After 2 to 4 hours the mixture was filtered and the residue in the filter was washed repeatedly.

SUGARS.—The extracts employed for sugar analysis were cleared as stated above and the method of Bertrand as presented by KERTESZ (**23**) was used for the determination of reducing and total sugars. Sucrose was hydrolyzed with invertase.

Experimental results

The experimental results are presented in two sections: (1) the chemical composition of the root exudates, and (2) the chemical composition of the root tissues.

CHEMICAL COMPOSITION OF ROOT EXUDATES

The data from many experiments summarized in table II show that the

TABLE II

DISTRIBUTION OF DIFFERENT NITROGENOUS FRACTIONS IN THE EXUDATE FROM ROOTS OF *PANDANUS VEITCHII* GROWN IN SOLUTION CULTURES WITH EITHER AMMONIUM OR NITRATE SALTS OR IN THE TOTAL ABSENCE OF AN EXTERNAL SUPPLY OF NITROGEN

NITROGENOUS FRACTIONS	NUTRIENT SOLUTIONS		
	AMMONIA	NITRATE	MINUS-NITROGEN
Inorganic			
Ammonium	0.013	0.002	0.003
Nitrate	0.000	0.030	0.000
Soluble organic			
Glutamine	0.008	0.003	0.002
Asparagine	0.016	0.016	0.006
Mono-amino	0.162	0.135	0.063
Basic	0.077	0.048	0.038
α-amino	0.212	0.171	.....
Total organic	0.255	0.194	0.109

exudates of the roots grown with ammonium nutrition contained considerably greater quantities of total nitrogen than those grown in the nitrate or minus-nitrogen cultures. They also show that the roots of the nitrate series contained more total nitrogen than those which lacked an external nitrogen supply. The presence of organic nitrogen in the exudates of the roots grown in the minus-nitrogen solutions is exceedingly interesting because it indicates that translocatory nitrogen, derived by hydrolysis of reserve proteins, may be present in fair quantities in the sap of plants grown in minus-nitrogen cultures. Of the different fractions of organic nitrogen alpha-amino nitrogen is the greatest. As it is included mostly in the fractions indicated as monoamino and basic nitrogen the difference between the value of the sum of the latter two fractions and that of the former is small indicating that other nitrogenous fractions are present in extremely small quantities. Amide either as glutamine or asparagine occurs in small amounts. Occasianal exceptions may be found, as in table III, where this fraction is relatively high.

TABLE III

DISTRIBUTION OF DIFFERENT NITROGENOUS FRACTIONS IN EXUDATE FROM ROOTS OF *PANDANUS VEITCHII* GROWN IN SOLUTION CULTURES WITH EITHER AMMONIUM OR NITRATE NITROGEN OR IN THE TOTAL ABSENCE OF AN EXTERNAL SUPPLY OF NITROGEN

NITROGENOUS FRACTIONS	NUTRIENT SOLUTIONS		
	AMMONIA	NITRATE	MINUS-NITROGEN
Inorganic			
Ammonia	0.005	0.007	0.005
Nitrate	0.000	0.135	0.000
Soluble organic			
Amide	0.200	0.055	0.017
Amino	0.490	0.163	0.045
Total organic	0.690	0.218	0.062

Some of the conditions which favor the formation of great quantities of amide nitrogen will be mentioned in the discussion.

Ammonium occurs in very small amounts in the exudate regardless of the kind of nitrogenous salts employed in the nutrient solution because of the great rate of assimilation of this fraction of nitrogen by the root tissues (**9, 64, 65, 72, 73**). The presence of ammonium, in traces, in the exudate of roots grown in nitrate-containing or minus-nitrogen cultures is due possibly in large part to hydrolyzed proteins. That nitrate might have been reduced to ammonium in the nitrate cultures is also probable. The quantities of ammonium found in the nitrate-supplied plants, however, are small and of the

same magnitude as those found in the minus-nitrogen cultures, but, keeping in mind the great rate at which ammonium is assimilated, its presence in more than traces would scarcely be anticipated in either case (**9, 64, 65, 72, 73**).

Nitrate may be found in small or large quantities in the exudate of roots owing respectively to an increased or decreased rate of assimilation of this fraction. In tables II and III and figures 2 and 3 it is shown that there was found from 30 to 135 mg. of nitrate nitrogen per liter of exudate. The variations are possibly due to differences in the physiological conditions of the root cells which may favor the reduction and assimilation of nitrates more at certain times than at others. Similar observations have been made in other plants (**15, 70**).

A point which deserves further emphasis in these studies is the alpha-amino nitrogen which, in nearly all cases, is between 70 and 90 per cent. of the total soluble organic nitrogen in the exudate. No protein either coagu-

FIG. 1. *Pandanus veitchii* shoot with roots of different lengths and diameters but without rootlets which are only produced when the tip is either placed in a nutrient solution or enters the soil.

lable by heat or filterable could be found in the exudate in more than mere traces.

Chemical composition of root tissues

Distribution of nitrogenous fractions in the tissues of roots grown in ammonium or nitrate cultures.—The roots grown in the different cultures were cut, as stated previously, into different parts separating the lateral and terminal rootlets from the main root tissues. Also the tissues of the terminal fraction of the main root were separated from those of the intermediate and proximal region as illustrated in figure 1. The basal tissues of

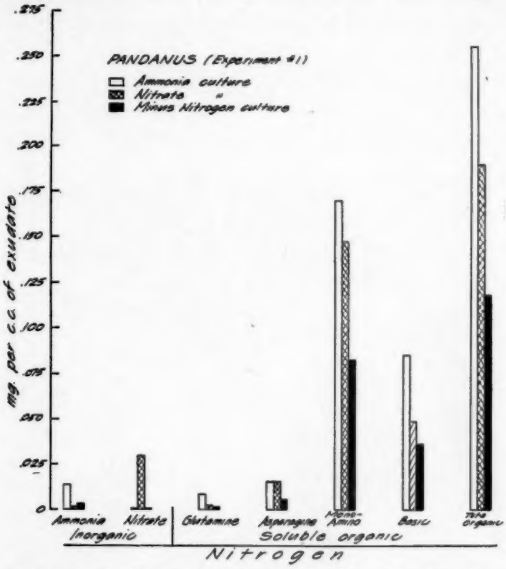

Fig. 2. Distribution of different fractions of inorganic and soluble organic nitrogen in the root exudates of *P. veitchii* grown in ammonium-, nitrate- or minus-nitrogen-solution cultures.

the roots, *i.e.*, those in close proximity to the stem, were not used. A comparative examination of the data in table IV and in figures 4, 5, and 6, with respect to the distribution of the different nitrogenous fractions in the stele and cortex of various regions of the root, discloses the following facts:

Distribution in the rootlets as contrasted in figure 4.—1. The amounts of nitrate in the rootlets of the nitrate-supplied plants were considerably higher than the ammonium nitrogen in the rootlets of the ammonium series. These findings are indicative of a lower rate of assimilation of nitrate as compared to ammonium in the rootlet tissues of *Pandanus veitchii*.

2. The glutamine in the rootlets of the ammonium cultures is considerably greater than in those of the nitrate group, indicating a greater rate of glutamine synthesis from ammonium than from nitrate ions.

3. The amounts of asparagine are about the same in the rootlets of both ammonium and nitrate cultures.

4. The quantities of soluble mono-amino nitrogen are greater in the rootlets of the plants grown in ammonium than in those receiving nitrate, while soluble basic nitrogen is of about the same magnitude in both cases.

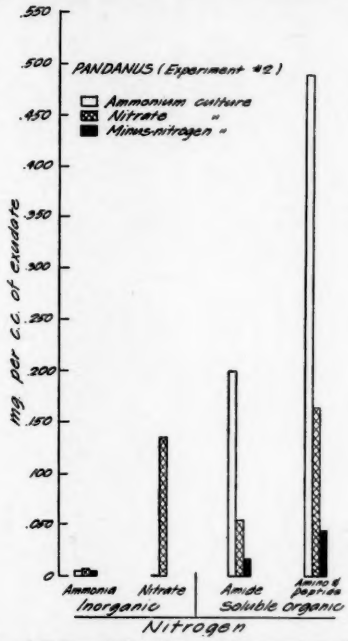

FIG. 3. Distribution of different fractions of inorganic and soluble organic nitrogen in the root exudates of *P. veitchii* grown in ammonium-, nitrate- or minus-nitrogen-solution cultures.

5. The nitrogenous fractions of insoluble nitrogen, with the exception of mono-amino nitrogen, are about the same in the rootlets of the plants of both ammonium and nitrate cultures.

DISTRIBUTION IN THE TERMINAL TISSUES OF THE MAIN ROOT AS CONTRASTED IN FIGURE 5.—1. As in the rootlets nitrate is considerably higher in the terminal tissue of the main root of the nitrate-supplied plants than is ammonium in comparable tissues of ammonium-grown plants. Nitrate is present only in the roots grown in nitrate cultures, whereas ammonium nitrogen

TABLE IV

DISTRIBUTION OF DIFFERENT NITROGENOUS FRACTIONS AS MG. NITROGEN PER GRAM FRESH WEIGHT IN VARIOUS ROOT TISSUES OF *PANDANUS VEITCHII* GROWN IN NUTRIENT SOLUTIONS AND CONTAINING EITHER AMMONIUM OR NITRATE SALTS AS SOURCES OF NITROGEN

Nutrient solution	Nitrogenous fractions	Main root tissues						Rootlet tissues
		Proximal		Intermediate		Terminal		
		Cortex	Stele	Cortex	Stele	Cortex	Stele	
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Ammonium-N	Inorganic							
	Ammonium	0.005	0.006	0.004	0.015	0.008	0.019	0.038
	Nitrate	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Soluble organic							
	Glutamine	0.004	0.009	0.008	0.020	0.027	0.045	0.038
	Asparagine	0.090	0.123	0.068	0.133	0.138	0.164	0.127
	Mono-amino	0.436	0.365	0.517	0.667	0.576	0.929	0.770
	Basic	0.231	0.272	0.313	0.540	0.332	0.696	0.345
	Insoluble organic							
	Amide	0.024	0.048	0.057	0.094	0.036	0.116	0.035
	Mono-amino	0.149	0.141	0.113	0.222	0.128	0.228	0.132
	Basic	0.096	0.101	0.120	0.223	0.075	0.324	0.062
	Humin	0.201	0.236	0.201	0.286	0.156	0.353	0.156
Nitrate-N	Inorganic							
	Ammonium	0.004	0.010	.....	.....	0.008	0.025	0.006
	Nitrate	0.018	0.095	.....	.....	0.050	0.100	0.180
	Soluble organic							
	Glutamine	0.008	0.017	.....	.....	0.013	0.013	0.002
	Asparagine	0.050	0.182	.....	.....	0.145	0.352	0.124
	Mono-amino	0.541	0.552	.....	.....	0.586	0.863	0.667
	Basic	0.298	0.420	.....	.....	0.289	0.555	0.340
	Insoluble organic							
	Amide	0.033	0.135	.....	.....	0.100	0.296	0.031
	Mono-amino	0.050	0.174	.....	.....	0.125	0.385	0.053
	Basic	0.047	0.178	.....	.....	0.090	0.452	0.063
	Humin	0.115	0.239	.....	.....	0.290	0.481	0.151

occurs in the roots grown in cultures containing either ammonium or nitrate. The quantities of ammonium found under both conditions, however, are comparatively small apparently owing to rapid assimilation of ammonium. The quantities of nitrate in the tissues of the stele are greater than in those of the cortex, possibly, because the vessels of upward conduction are mostly located in the former tissues (**11, 32**).

2. Glutamine is more prevalent in the stele than in the cortex. The quantities found are greater in the roots grown in ammonium than in nitrate

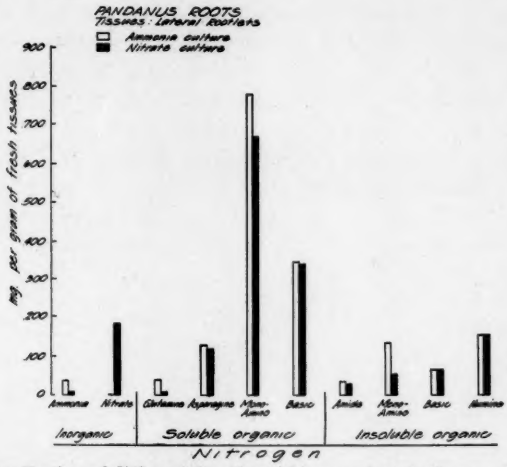

FIG. 4. Distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the rootlet tissues of plants grown in ammonium- or nitrate-solution cultures.

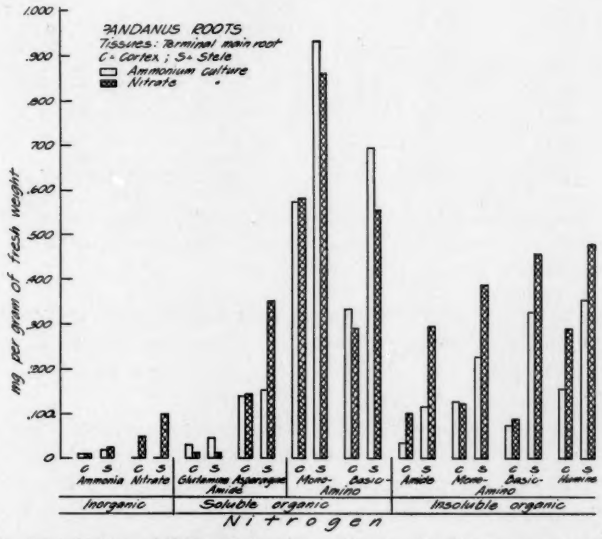

FIG. 5. Distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the cortex and stele tissues of the terminal section of the main root of *P. veitchii*.

cultures, which condition is associated with a greater rate of ammonium than of nitrate assimilation and also with conditions peculiar to ammonium metabolism which favor the synthesis of great amounts of glutamine as observed by CHIBNALL and WESTALL (7) and VICKERY, PUCHER, and CLARK (79).

3. Asparagine is distinctly more abundant in the stele than in the cortex. Differences between the amounts of asparagine of the roots of ammonium- and nitrate-grown plants are relatively great, indicating a greater rate of synthesis of asparagine from nitrate than from ammonium.

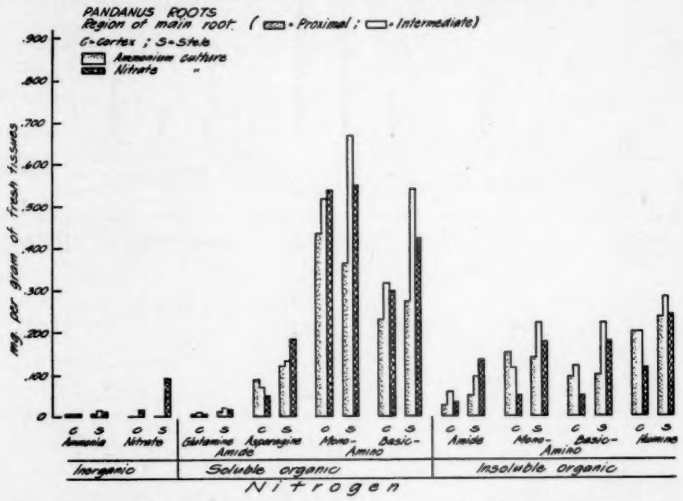

FIG. 6. Distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the cortex and stele tissues of the intermediate section of the main root of *P. veitchii*.

4. The amounts of mono-amino and basic soluble nitrogen are considerably greater in the stele than in the cortex. Also, the amounts of both fractions contained in the roots of plants grown in ammonium are considerably greater than those of plants supplied with nitrate.

5. The various fractions of insoluble nitrogen are higher in the stele than in the cortex. With one minor exception, the amounts of all the fractions of insoluble nitrogen of the roots grown in nitrate are higher than those of the roots furnished with ammonium, indicating that more protein nitrogen accumulates in the roots of nitrate than in those of ammonium-supplied plants.

DISTRIBUTION IN THE INTERMEDIATE REGION OF THE MAIN ROOT AS CONTRASTED IN FIGURE 6.—Analytical values of the intermediate tissues of the

main root are only available for the roots grown in ammonium cultures. These values, contrasted with those of figure 5, show a gradual decrease in the quantities of most nitrogenous fractions possibly owing to greater amounts of cellulosic material, vacuolation, and other such features associated with maturation.

The proximal tissues of the main root of the plants grown in nitrate cultures compare more in age and in general development with the intermediate than with the proximal tissues of the plants receiving ammonium. In comparative observations this point should be taken into consideration.

1. Ammonium in the tissues of roots of plants grown either in ammonium or in nitrate cultures is very low while nitrate in the root tissues of the latter cultures is relatively abundant.

2. Glutamine is very low in the root tissues of both lots of plants.

3. Asparagine is relatively abundant in the roots of plants of both ammonium and nitrate cultures. Certain differences in the content of asparagine in the proximal and intermediate tissues of the cortex cannot be explained satisfactorily.

4. Soluble mono-amino and basic nitrogen values in the tissues of the stele of nitrate-grown plants are lower with respect to the intermediate region of the main root, but higher with respect to the proximal fraction of plants grown in ammonium cultures. Certain variations in the mono-amino nitrogen content of the stele and cortical tissues cannot be explained.

5. The distribution of the different fractions of insoluble nitrogen in the intermediate region of the roots of the two different cultures shows many fluctuations. Comparing the cortex of either the proximal or intermediate portions of the roots grown in ammonium cultures with those of the nitrate series, with the exception of amide, the quantities of all the other fractions of insoluble nitrogen are higher in the roots of the former than in those of the latter series. The amounts of different fractions of insoluble nitrogen in the stele of both lots are greater than in those of the cortex. Also the proximal portion of the roots of the plants grown in ammonium cultures contain, with one exception, slightly smaller amounts of insoluble nitrogen than the intermediate region.

Distribution of sugars.—The distribution of sugars in the stele and cortex of different parts of the root, as presented in table V and figure 7, shows that:

1. In the main root total sugars occur in greater amounts in the stele than in the cortex. In the rootlets only very small quantities are found.

2. Reducing sugars occur in both stele and cortex. Their quantities in the stele increase, with minor exceptions, from the proximal towards the terminal portion of the main root, indicating that there is an accumulation of these sugars at the distal end of the main root possibly owing to a de-

TABLE V

DISTRIBUTION OF DIFFERENT SUGARS AS PERCENTAGE OF FRESH WEIGHT IN VARIOUS ROOT TISSUES OF *PANDANUS VEITCHII* GROWN IN NUTRIENT SOLUTIONS AND CONTAINING EITHER AMMONIUM OR NITRATE SALTS AS SOURCES OF NITROGEN

NUTRIENT SOLUTION	SUGAR FRACTIONS	MAIN ROOT TISSUES						ROOTLET TISSUE
		PROXIMAL		INTERMEDIATE		TERMINAL		
		CORTEX	STELE	CORTEX	STELE	CORTEX	STELE	
Ammonium nitrogen	Total	1.20	2.60	0.48	1.88	0.52	2.35	0.02
	Reducing	1.10	0.69	0.48	0.96	0.52	2.11	0.02
	Sucrose	0.10	1.91	0.00	0.92	0.00	0.24	0.00
Nitrate nitrogen	Total	0.90	3.31	.....	.....	0.35	1.64	0.04
	Reducing	0.90	1.10	.....	.....	0.35	1.06	0.04
	Sucrose	0.00	2.21	.....	.....	0.00	0.58	0.00

creased rate of translocation to the rootlets. With respect to the location in the cortex the quantities of reducing sugars increase from the terminal to the basal end of the main root. A comparison of the distribution of re-

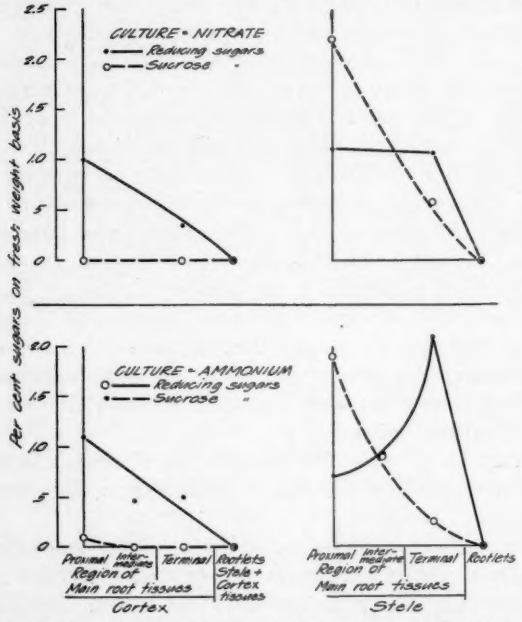

FIG. 7. Distribution of reducing sugars and sucrose in the rootlets and cortex and stele tissues of the terminal, intermediate, and proximal sections of the main root of *P. veitchii* grown in ammonium- or nitrate-solution cultures.

ducing sugars in the stele and cortex shows that within the same fraction of the root there is an indirect proportionality, in that the amounts of reducing sugars in the stele are high and those in the cortex are low. The rootlets contain only traces of these sugars.

3. Sucrose is limited almost exclusively to the stele, the cortex containing only traces or none. The quantities of sucrose in the stele increase from the terminal towards the basal end of the main root, apparently indicating that the conversion of reducing sugars to sucrose is a highly specialized function of the stele and that with a further upward movement of the reducing sugars into the stele their conversion into sucrose is more complete.

NITROGEN AND SUGAR DISTRIBUTION GRADIENTS.—In the three fractions of the main root the quantities of sugars are comparatively enormous as compared to those in the lateral rootlets. The exceedingly small amounts found in the rootlets may be attributed either to a rapid utilization or to a decreased rate of transport from the main root. The amounts of soluble organic nitrogen are almost as great in the rootlets as in the main root, although they should seemingly have been higher owing to their closer contact with the source of inorganic nitrogen. The amounts of insoluble nitrogen of the rootlets are also considerably smaller than those of the main root tissues, which should have been greater in the former than in the latter on the basis of cellular senility and vacuolation. Adequate reasons to explain all these differences cannot be offered without additional information. It is possible, however, to explain certain of the differences on the basis of anatomical variations between the tissues of the main root and those of the rootlets. The ratio of the area or volume of stele to cortical tissues in different fractions of main roots varying in diameter between 10 and 35 mm. ranges from 0.425 to 3 whereas in those rootlets varying in diameter from 2.5 and 10 mm. the range is from 0.114 to 0.425. A more comprehensive idea of the

TABLE VI

AREA IN SQUARE MILLIMETERS AND PERCENTAGE OF STELE AND CORTEX TISSUES IN CROSS SECTIONS OF ROOTS OF DIFFERENT DIAMETERS

ROOT CROSS SECTION		STELE		CORTEX	
DIAMETER	AREA	AREA	PERCENTAGE OF TISSUE	AREA	PERCENTAGE OF TISSUE
mm.	*mm.*^{2}	*mm.*^{2}	%	*mm.*^{2}	%
37.0	1076.0	858.0	79.6	218.0	20.4
13.0	133.0	50.0	37.5	83.0	62.5
8.0	50.0	12.5	25.0	37.5	75.0
5.0	19.7	3.2	15.8	16.5	84.2
2.5	4.9	0.5	10.0	4.4	90.0

area occupied by stele and cortical tissues in a cross section of roots of different diameters may be obtained from table VI, where such areas are recorded in square millimeters, and also from figure 8 where the areas occupied have been plotted on a percentage basis.

Our detailed analytical data of the stele and cortical tissues of the main root indicate, with a few minor exceptions, that the amounts of nearly all fractions of nitrogen in the stele are considerably higher than in the cortex. One may conclude, on the basis of the analytical and anatomical data, that the relatively low values of the different fractions of nitrogen in the rootlets are due, in a great measure, to a predominance of cortex over stele. With

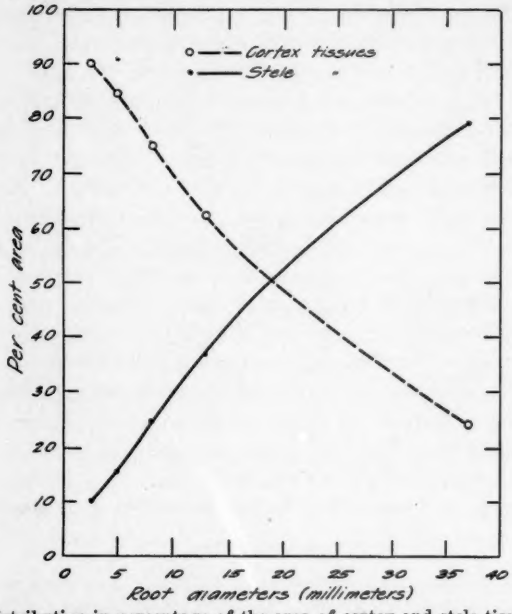

FIG. 8. Distribution in percentage of the area of cortex and stele tissues in the roots of *P. veitchii* varying in total diameter.

respect to the distribution of sugars in the tissues of the rootlets very little can be said because in the compound samples of both stele and cortex these substances were found only in traces.

There are decided gradients of both nitrogen and sugars between the distal and proximal regions of the main root. The quantities of inorganic and soluble organic nitrogen and proteins are greater in the terminal than in the intermediate fractions of the root. The amounts of reducing sugars are high in the intermediate and low in the terminal tissues of the cortex of

the main root, but these values are reversed in the stele of the same regions because of the conversion of reducing sugars to sucrose.

Discussion

Ammonium assimilation by the roots of *Pandanus veitchii* is very rapid if not instantaneous. Very small quantities of ammonium are nearly always present in the tissues and probably result in most cases from hydrolyzed proteins. At least it is present in comparable concentration even though there is no external nitrogen supply available (figs. 2, 3). The evidence that ammonium is assimilated instantaneously can be best obtained from root exudates, as the tracheal sap under such conditions contains very little if any proteins or materials of injured or crushed cells which may serve as sources of ammonium from hydrolyzed proteins.

The immediate stable products of ammonium assimilation, which are found in appreciable amounts in the tissues, are amino acids, glutamine, and asparagine. The various biochemical reactions which have preceded the synthesis of these compounds are unknown. Glutamine has been found by CHIBNALL and WESTALL (**7**), VICKERY, PUCHER, and CLARK (**79**) to be produced in great amounts in plant tissues after ammonium assimilation. In the present case the amounts of glutamine obtained in the exudate and in the tissues of the roots were relatively small indicating that either the concentrations of ammonium supplied in the nutrient solution were comparatively low or that the metabolic mechanism of *Pandanus veitchii* is such as to produce more amino instead of amide nitrogen from ammonium, a condition observed by SCHWAB (**63**) in many plants.

Nitrate is not assimilated as readily as ammonium by the root tissues of *Pandanus veitchii* as comparatively great amounts pass through the tissues entirely unaltered and are found in the exudate. The relatively slower rate of nitrate assimilation, as compared with that of ammonium, may be due to the various intermediary but highly necessary reduction reactions preceding assimilation, the velocity of which varies considerably in different species and under different conditions. With certain plants nitrate may be assimilated, under highly favorable conditions, entirely in the roots whereas with other plants nitrate assimilation may be conducted mostly in the leaves (**20, 35**). With respect to *Pandanus veitchii*, where oxygen supply (due to aeration) and carbohydrates were plentiful, the decreased rate of assimilation may be attributed to some inherent causes or to factors of which we have very little comprehension. Experimental results (unpublished) indicating a very low rate of nitrate assimilation by pineapple roots and a higher one by the leaves of the same plant have been obtained by the writers. Woo (**81**), as well as others, has obtained similar evidence for other plants.

The amounts of asparagine formed during the assimilation of nitrate, particularly in the tissues of the stele, are considerably greater than those obtained during ammonium assimilation. Whether the oxygen atoms in the nitrate molecule are responsible for the formation of greater amounts of asparagine or not, remains to be shown by additional experimentation.

Ammonium more than nitrate, owing to its greater degree of assimilability (**7, 9, 13, 26, 64, 65**), produces larger quantities of such fractions of soluble organic nitrogen as mono-amino and basic nitrogen at the terminal region of the main root. The quantities of similar fractions of soluble organic nitrogen appear to increase more in the intermediate tissues of the main root of plants grown in nitrate than in the comparable region of those grown in ammonium cultures. This condition may be due to the gradual assimilation of greater amounts of nitrate toward the proximal end of the root where sucrose is more plentiful.

The different fractions of protein or insoluble nitrogen are greater in the roots grown in nitrate than in ammonium cultures. However, no explanation thoroughly satisfactory and unbiased can be offered of this phenomenon. The writers' tentative opinion is that plants grown in nitrate maintain, even under optimal conditions, a relatively low rate of amino acid synthesis. Carbohydrates (and possibly other substances, whatever these may be) essential for protein synthesis are therefore available in sufficient amounts, and proteins are produced regularly and without interruption at all times. But with plants grown in ammonium, under favorable conditions, there is a high rate of organic nitrogen synthesis. In consequence, carbohydrates (and possibly other substances, whatever they may be) essential for protein synthesis are depleted very rapidly in the formation of amino acids. In the absence of these substances the rate of synthesis of proteins decreases and the more readily formed products of soluble organic nitrogen accumulate in the tissues in great amounts. This suggestion does not mean that the synthesis of proteins from nitrates is direct nor does it contradict a long accepted theory (**27**) that proteins are formed from amino acid or amide nitrogen. BARTON-WRIGHT and MCBAIN (**5**) have observed a somewhat similar condition in the potato and advanced the theory that protein nitrogen is formed from nitrate nitrogen by the direct conversion of the latter first into proteose nitrogen and then into protein. DIKUSSAR (**13**), in his studies on the assimilation of nitrite nitrogen by higher plants, has presented evidence in which he compares values of absorbed nitrogen from nutrient solution containing nitrite, nitrate, or ammonium, showing that with small amounts of ammonium, nitrate, or nitrite in the culture solution very high yields of protein nitrogen in plant tissues can be obtained. With great amounts of ammonium, nitrate, or nitrite in the culture solution the amounts of soluble organic nitrogen in the tissues, compared on a percent-

age basis, increase at a greater rate than those of protein nitrogen. Certain of his values are reproduced in table VII to illustrate this condition.

TABLE VII

TOTAL NITROGEN AND PERCENTAGE OF PROTEIN AND SOLUBLE ORGANIC NITROGEN OF PLANT TISSUES ABSORBED FROM NITRITE, NITRATE, OR AMMONIUM SOLUTION CULTURES CONTAINING DIFFERENT AMOUNTS OF NITROGEN.

(From DIKUSSAR'S table III)

NITROGEN IN NUTRIENT SOLUTIONS	NITRITE			NITRATE			AMMONIUM		
	TOTAL	PROTEIN	SOLUBLE	TOTAL	PROTEIN	SOLUBLE	TOTAL	PROTEIN	SOLUBLE
mg./l	*gm.*	%	%	*gm.*	%	%	*gm.*	%	%
60	0.438	52.0	48.0	0.306	60.8	39.2	.....	.....	.....
40	0.396	57.0	43.0	.....	.....	.....	0.546	44.3	55.7
20	0.247	72.8	27.2	0.227	66.5	33.5	.....	.....	.....
10	0.291	75.9	24.1	0.286	74.4	25.6	.....	.....	.....
5	0.286	74.4	25.6	.....	.....	.....	0.283	76.7	23.3

The experimental values of DIKUSSAR are in harmony with our tentative hypothesis that the degree of assimilability and quantities of inorganic nitrogen coupled with the amounts of other substances essential for amino acid and protein synthesis are factors responsible for the great differences found in the ratio values of protein : soluble organic nitrogen of plants grown respectively in nitrate and ammonium solution cultures.

With respect to substances necessary for protein synthesis the literature points out quite decidedly that an abundance of readily available carbohydrates is highly essential. SUZUKI (**66, 67, 68**) and MUENSCHER (**37**) have clearly demonstrated that in the presence of carbohydrates and inorganic nitrogen chlorophyllous plants are able to synthesize soluble organic and protein nitrogen. That besides leaves other plant organs such as roots are able to synthesize proteins either in the presence or absence of light has been demonstrated by MÜLLER-THURGAU (**38**), IWANOFF (**21**), and BAMBACIONI (**3**). IWANOFF, working with the roots of *Brassica napus, Daucus carota,* and *Solanum tuberosum,* observed that for the synthesis of protein in the absence of light his plants required a small amount of protein and a great supply of both amides and readily assimilable carbohydrates. BAMBACIONI on the basis of analysis of two sets of plants of *Vicia faba, Ricinus communis,* and *Cucurbita pepo,* one grown in plus-nitrogen and the other in minus-nitrogen solution cultures, concluded that all parts of the plant, and particularly the roots, are capable of synthesizing proteins. That carbohydrates are highly essential for protein regeneration in newly forming tissues of plants deprived of nitrogen and obtaining their nitrogen supply from the hydrolyzed

proteins of old tissues has been well demonstrated by PRIANISCHNIKOW (**53**). There is also undisputable evidence (**46, 53**) that proteins stored in plant tissues undergo hydrolysis at a much greater rate in the absence than in the presence of carbohydrates.

Yet there are certain cases known where besides carbohydrates certain other factors, of which our knowledge is limited, influence the rate of protein synthesis. LOOSE and PEARSALL (**28**) claim, from certain experimental evidence they have obtained on *Chlorella*, that the velocity of protein synthesis is five times greater in the light than in darkness though the final equilibrium is similar in both cases if sufficient carbohydrates are available. It is rather unfortunate that these investigators have not reported in their results the amounts of readily assimilable carbohydrate in the tissues of their plants or those in the nutrient solution at the end of the experiment to show additional relationships between carbohydrates and protein synthesis.

Other conditions, and particularly deficiencies of certain mineral elements, although acting indirectly, are capable of reducing the rate of protein synthesis. The work of NIGHTINGALE, SCHERMERHORN, and ROBBINS (**47**), HARTT (**17**), RICHARDS and TEMPLEMAN (**62**) with potassium-deficient plants shows quite conclusively that protein synthesis is retarded in insufficient amounts of potassium. Similar effects have also been reported in plants grown in nutrient solutions deficient in certain other elements besides potassium. The direct effects of available carbohydrates in protein synthesis are well appreciated and easily understood, while the indirect effects of other factors, such as we obtain in mineral deficiencies, complicate this problem to a certain extent.

The results obtained on the distribution of reducing sugars and sucrose in the tissues of the stele and cortex are very interesting. The more or less complete absence of sucrose, and the singular presence of reducing sugars in the tissues of the cortex are indicative of sucrose being a storage and not a transport sugar. Moreover, the inverse proportionality which exists between the quantities of sucrose and reducing sugars in the stele and the greater amounts of sucrose in the proximal than in the terminal tissues indicate that reducing sugars enter the distal portion of the stele through the terminal tissues of the cortex of the main root where they are gradually converted into sucrose. The amounts of sucrose in the proximal region of the stele are higher while those of reducing sugars are lower, showing that the accumulation of sucrose in more mature cells is greater and the conversion of reducing sugars to sucrose more complete. The findings of COLIN (**10**) on the formation and distribution of sucrose in the sugar beet indicate that, although sucrose is formed in the leaves in sunlight, it is converted into reducing sugars during transportation into the root and there transformed again into sucrose. The experimental evidence of COLIN shows that the quantities

of sucrose diminish from leaves to stem. With respect to the migration of sucrose from the root towards the aerial parts, in two-year-old plants, COLIN states that sucrose leaves the tissues of the root as sucrose but as it approaches the aerial organs it is converted gradually into reducing sugars.

BARTON-WRIGHT and MCBAIN (**4**) have observed sucrose accumulation in the laminae of diseased crinkle plants, while hexose was abundant in the healthy and starch had fallen to low values in both diseased and healthy plants. Their interpretation of these results, with respect to healthy leaves, is that hexoses are the ultimate products of mostly sucrose and of very little starch hydrolysis and that sucrose is transported out of the leaves to the tubers; whereas with diseased plants sucrose formation is taking place directly and mostly from starch and only to a very small extent from hexose, but that sucrose is the sugar of transport in both cases. Our data do not agree very well with the interpretations of BARTON-WRIGHT and MCBAIN unless we assume an upward transport of sucrose through the tissues of the stele in a manner analogous to that described by MASON, MASKELL, and PHYLLIS (**32**) with respect to stored nitrogen in which case they state as follows: ". . . the upward movement of stored nitrogen may be an example of upward movement via the phloem in opposition to the downward movement of carbohydrates, but that until more is known as to the conditions determining liberation of stored nitrogen into the tracheae the alternative of upward movement entirely via the xylem cannot be excluded." In a preceding paper MASON and MASKELL (**31**) state that ". . . when wood and bark are separated movement of sugar takes place through the bark at nearly the normal rate and that the downward transport of carbohydrates occurs in the bark." Our data in table II support the claims of CURTIS (**11**) and of MASON, MASKELL, and PHYLLIS (**32**) that there is an upward movement of stored nitrogen through the wood tissues and that reducing sugars are transported through the tissues of the bark as indicated in table V.

The prominent position which sucrose occupies in the stele and its complete absence in the cortex cannot be overlooked. If there is any transport of sucrose at all it will be in an upward direction through the tissues of the stele and probably in the manner indicated by COLIN (**10**). The tissues of the stele are, from the point of view of sucrose deposition, comparable to the root tissues of the sugar beet as presented by COLIN. In the absence of more information on the nature of transport of sucrose we postpone any further discussion until additional experimental evidence is obtained.

Summary

A series of experiments was undertaken in order to obtain information on the absorption and assimilation of ammonium and nitrate nitrogen by

analyzing the exudate and the stele and cortex of different fractions of the excised roots of *Pandanus veitchii* grown in ammonium, nitrate, and minus-nitrogen solution cultures respectively. The root tissues, but not the exudate, were also analyzed for reducing sugars and sucrose. The results were as follows:

1. Ammonium is assimilated instantaneously and at a high rate as it enters the root, where it is converted either into amide or amino nitrogen or more gradually into protein.

2. Nitrate is not assimilated as rapidly as ammonium and the amounts of the various fractions of soluble organic nitrogen are not as high, particularly in the terminal root tissues, as those found usually in roots grown in ammonium cultures.

3. The amounts of protein or insoluble nitrogen are greater in the roots of the plants grown in nitrate than in those receiving ammonium nutrition. Our tentative explanation of this condition is that roots grown in ammonium cultures, through the rapid formation of amino acids utilize and exhaust very rapidly the supply of carbohydrates and possibly of other substances essential for protein synthesis. With roots of plants grown in nitrate cultures, the rate of nitrate assimilation and synthesis of amino acids being comparatively low, carbohydrates and other substances essential for protein synthesis are not depleted but are always present in sufficient amounts to promote protein synthesis, the ultimate stage of nitrogen metabolism.

4. Organic nitrogen, newly synthesized in the roots, either from ammonium or nitrate, is transported through the tissues of the stele to the proximal regions of the root, following the path of water and mineral salt conduction in the roots. The channels which the hydrolytic products of stored nitrogen follow are the same, if our interpretation of the results obtained in minus-nitrogen nutrient cultures is correct. Certain of these studies made on root exudates indicate that, except for a great difference in the amounts of soluble organic nitrogen between plus-nitrogen and minus-nitrogen cultures, no other lines of demarcation can be drawn with our present analytical technique between newly synthesized and stored fractions of soluble organic nitrogen.

5. No proteins, in any appreciable quantity, were found in the exudate of roots, indicating that assimilated nitrogen is translocated as soluble organic and not as insoluble organic or proteinaceous nitrogen.

6. The distribution of reducing sugars and sucrose in the tissues of the stele and cortex shows that sucrose is present in great amounts in the stele but is lacking almost completely in the cortex. The data also suggest that reducing sugars, possibly, enter the tissues of the stele through the terminal tissues of the cortex of the main root. The amounts of reducing sugars in the stele are greatest at the point of entrance or terminal region, but de-

crease in the upper regions of the stele. At different levels along the root there is in the stele an indirect relationship between the amounts of reducing sugars and sucrose. In the terminal portion of the stele, which may serve as paths of entrance, reducing sugars are higher than sucrose, but in the proximal region of the main root, sucrose increases very rapidly in the stele, whereas reducing sugars decrease in about the same ratio, apparently indicating that there is a gradual but rapid conversion of reducing sugars to sucrose.

7. The data suggest that reducing sugars are the sugars of downward transport and that sucrose is stored in the stele. If sucrose ever enters the transport stream in the roots of *Pandanus veitchii* it may follow a course similar to that in the sugar beet, that is, an upward instead of a downward direction. The tissues of the stele of the roots of *Pandanus veitchii*, on account of their ability to synthesize and store sucrose, are comparable to the root tissues of the sugar beet, as discussed by COLIN.

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EFFECT OF ENVIRONMENTAL FACTORS UPON THE COLOR OF THE TOMATO AND THE WATERMELON[1]

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(WITH SEVENTEEN FIGURES)

Introduction

A contribution to the knowledge of the physiological mechanism involved in the production of color in fruits of commercial value is of considerable advantage and usefulness at the present time. A premium is always paid for desirably colored products, both in the canning and in the fruit and market gardening industries. The natural color of ripe fruits may be due to pigments of very diverse nature, produced by the protoplast either in the living plastid, in the cell sap, or deposited in the cell wall. The plant physiologist is particularly interested in the biochemical changes which are responsible for the development of these varied pigments, the conditions under which they are produced, and their ultimate fate in the animal body. At the present time the layman is fast becoming familiar with the fact that certain of these yellow plant pigments are precursors of vitamin A and play an important rôle in human nutrition.

The chlorophyll of higher plants is produced in special organs of the living cell called chloroplasts. According to ZIRKLE (**63**) first mention of these "green granules" was made by COMPARETTI (**13**) in 1791. The living nature of these bodies, however, was not established until almost a century later when SCHIMPER (**51**) proved that they arise by division of preexisting structures and gave them the name "plastids." The pigments of the green leaf were first extracted and given the name "chlorophyll" by PELLETIER and CAVENTOU (**50**) in 1818, but it was not until 1864 that the first separation of the chlorophyll complex into green and yellow components was made by STOKES (**55**). In a paper before the Royal Society he stated: "I find the chlorophyll of land plants to be a mixture of four substances, two green and two yellow, all possessing highly distinctive optical properties. The green substances yield solutions exhibiting a strong red fluorescence; the yellow substances do not." It is to this man that science owes the discovery of the still widely used method of partition between two immiscible solvents. He stated definitely, "For convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation." Unfortunately, this discovery was overlooked by

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plant physiologists and did not come into general use until after BORODIN (**11**) isolated two different types of crystals from the yellow constituent. In 1906 TSWETT (**57**) introduced the chromatographic method for the separation of the component pigments, which also is widely used today. Furthermore, it was TSWETT who introduced the term "carotenoid" and proposed grouping the several yellow pigments found in leaves under this name. ARNAUD (**9**) some years previously had investigated the yellow pigment accompanying chlorophyll and discovered that it was identical with the carotene isolated from the carrot (*Daucus carota* Linn.) by WACKENRODER (**58**) in 1831. ARNAUD first established the hydrocarbon nature of the pigment and proposed a relationship between the yellow pigments and chlorophyll, suggesting a rôle for them similar to that of hemoglobin. It is interesting to note that recently JAVELLIER, ROUSSEAU, and EMERIQUE (**28**) examined one of ARNAUD's preparations sealed in a tube of hydrogen and found it to possess biological activity after a period of forty years.

In 1913 MONTEVERDE and LUBIMENKO (**44**) presented a spectrocolorimetric method for the estimation of plastid pigments and studied their transformation in living plant tissue. In the same year WILLSTÄTTER and STOLL (**61**) applied the method of splitting a complex molecule into cleavage products. By purification and further study of these products they were able to reconstruct the chlorophyll molecule and by this means obtained proof of its constitution. During the course of these investigations WILLSTÄTTER and MIEG (**60**) obtained the two yellow pigments as secondary products in extracting chlorophyll from nettle leaves and established their empirical formulae. The observation that carotenoids may act as oxygen carriers or absorbers led WILLSTÄTTER to introduce this concept into the photosynthesis mechanism, as a means of reducing chlorophyll b and keeping the a and b components in equilibrium. In 1927 ZECHMEISTER (**8**) showed by catalytic hydrogenation that carotene contained eleven ethylenic linkages and was a bicyclic compound. Lycopene was found by the same investigator to contain thirteen double bonds and to be an unsaturated aliphatic molecule. Lycopene was first discovered in the fruit of the tomato (*Lycopersicum esculentum* Mill.) by MILLARDET (**40**) in 1876, although it did not receive the name lycopene until 1903 when SCHUNCK (**52**) presented a paper before the Royal Society of London. WILLSTÄTTER and ESCHER (**59**) isolated this red pigment from Italian tomatoes in 1910 and suggested its hydrocarbon nature. Today both carotene and lycopene are recognized as polyene hydrocarbons and PALMER (**49**) suggests a new system of nomenclature for the entire group. Furthermore, KUHN (**32**) has shown that the color of certain carotenoid compounds depends upon a chain of more than four conjugated double bonds and presented a method of separation of carotenoid pigments which has been developed further by MILLER (**41**). The fact that the smell

of violets was apparent on the oxidation of carotene led KARRER (**3**) to believe the odor due to the presence of β-ionone rings. Subsequent investigation proved the symmetrical structure of carotene and that both ends of the hydrocarbon chain were cyclic and identical. Lycopene was found to add thirteen molecules of hydrogen, forming perhydrolycopene and by a series of reactions lycopene was shown to be a symmetrical acyclic hydrocarbon carrying thirteen double bonds, eleven of which are conjugated. The deep color of lycopene adds weight to KUHN's (**33**) observation on crocetin and bixin that deep color requires more than four conjugated double bonds. Furthermore, KARRER accomplished the difficult synthesis of perhydrovitamin A and then was able to present the structural formula of vitamin A. Previous to this time OSBORNE and MENDEL (**48**) and MCCOLLUM and DAVIS (**38**) had found the A factor to be present in cod liver oil and in butter. STEENBOCK (**54**) brought out the fact that only products containing certain yellow pigments had vitamin A potency. DRUMMOND (**17**) repeated the work using pure carotene and obtained negative results due to the solvent used. It remained for EULER (**2**) and KARRER (**3**) independently of each other, to harmonize these apparently discordant results and to prove β-carotene to be the parent substance which is converted to vitamin A by the animal body. Numerous contributions by these and other investigators have shown the existence in plants of a series of polyene hydrocarbons, a series of polyene alcohols, and several polyene carboxylic acids. The carotenoid pigments are of wide distribution in nature and are responsible for a great number of colors in all plant organs. They are known to exist together and in combination with other organic pigments, so that fruit or flower color is likely to consist of a pigment complex, variation being due to the balance between the various pigments involved.

The marked effect of temperature upon the production of lycopene was first discovered by DUGGAR (**18**) in 1913. This remarkable contribution showed conclusively that a temperature of 30°–37° C. clearly inhibited the development of lycopene both in detached fruit and in fruit growing on the vine. The factors for reddening were not destroyed by the high temperature, for upon return to a temperature of 20° C. lycopene formation proceeded rapidly. Lycopene formation follows the destruction of the chlorophyll but certain other changes which take place within the cells remain unknown. Lycopene formation, however, does not necessarily follow decomposition of chlorophyll as HARVEY (**27**) has recently shown. The chemical reactions and the relationship between the pigments, the source from which the pigments are derived, the physiological importance of each component, are questions which remain little understood and for which quest is being carried on throughout the world. Lycopene formation occurs only at temperatures above 10° C. and below 37° C. Chlorophyll decomposition

requires a temperature above 15° C. and is removed less rapidly at 40° C. than at 24° C. In addition, HARVEY (**26**) has shown that chlorophyll decomposition by ethylene can occur only when the cells are in an active metabolic state. The presence of fungi, slight mechanical injuries, or certain other conditions prevent the balancing action of ethylene. Therefore, it is likely that the decomposition of chlorophyll does not take place by a single enzyme mechanism, but that the whole actively metabolic system of the cell is required. The same is indicated for lycopene formation.

Extensive reviews of the literature are found in monographs by KARRER (**3**), ZECHMEISTER (**8**), LEDERER (**4**), MAYER (**5**), BERGMANN (**1**), and PALMER (**6**).

Determination of color

The subject of color is a most fascinating one for scientist and layman alike. Man has been color-conscious for a long time, the word itself being very old. Like many old words, the word "color" has been used to convey different meanings. As a household word, its meaning is vastly different from its use by the physicist.

Color depends on the constituents of white light which are not absorbed but which are reflected. Color sensations in the normal individual depend on the responses of the retina of the eye to different wave lengths of light. If the light has one definite wave length, the color produced will have a pronounced hue. When Sir ISAAC NEWTON passed white light through a glass prism, he obtained a continuous spectrum containing all the spectral hues, which may be described as red, orange, yellow, green, blue, and violet. However, NEWTON recognized their objective physical meaning, saying plainly, "The rays, to speak properly, are not coloured. In them is nothing else than a certain power or disposition to stir up a sensation of this or that colour. . . . So colours in the object are nothing but a disposition to reflect this or that sort of rays more copiously than the rest." NEWTON also knew that any color could be matched by mixing the component blue, green, and red portions in proper proportions. If the primary spectral colors, *i.e.*, light of wave lengths of 460, 530, and 650 mμ be mixed in varying proportions, any hue whatsoever can be produced. The visible spectrum is arbitrarily divided into six broad regions (fig. 1), but the color of the spectrum varies continuously throughout its length. The physical difference between red, green, or blue is one of wave length. The unit commonly used for specifying this difference is the millimicron. Table I shows the approximate wave lengths of these six broad regions.

In the analysis of color the eye is not a good analytical instrument. It gives a confused sensation owing to the inability clearly to distinguish between the three components of color, particularly brightness and purity. When a color is measured spectroscopically, such confusion is eliminated.

TABLE I

SPECTRAL REGIONS IN TERMS OF WAVE LENGTH

	$m\mu$
Violet	400–450
Blue	450–500
Green	500–570
Yellow	570–590
Orange	590–610
Red	610–700

If the light from a reflected surface is measured by a colorimetric method, the eye perceives only those wave lengths to which it is sensitive. Different eyes are sensitive in different degrees to different wave lengths. In such a procedure different observers may obtain different results. Also, when a color is observed under varying quality of illumination, the sensation produced under each light will be different. Colorimetric measurements, therefore, are not dependent on the light source alone, but also on the idiosyncrasies of human vision. Spectrophotometry, on the other hand, provides a method for completely specifying the stimulus of color independent of material color standards, or of abnormalities of the observer's vision and other personal eccentricities. The color of a reflecting surface can be determined with sufficient precision, and the analysis of any color can be plotted in the form of a spectrophotometric curve, as in figure 1. Thus, two or more colors may be compared by comparing their curves, each of which constitutes a permanent record of the color and does not require the maintenance of a sample of the color.

The color of green tomato fruits is due to the complex chlorophyll mixture within the chloroplast. The fact that yellow pigments accompany chlorophyll has been known since the fundamental work of STOKES in 1864. Upon the decomposition of chlorophyll at temperatures from 30°–37° C., carotenes, xanthophylls, and certain yellow pigments give the tomato its characteristic color. If the temperature during the ripening period is held below 30° C., unknown changes take place within the actively metabolic cells, resulting in the formation and accumulation of lycopene, the carmine-red isomer of carotene. The color changes due to the physical mixture of the various pigments can be satisfactorily determined by spectrophotometric reflection measurements. Thus, optics furnishes the plant physiologist with an accurate method of color measurement, and enables him to interpret color changes owing to the fact that there are two or more pigments present in physical mixture rather than in chemical combination.

In this work reflection measurements from the surface of the fruit were taken with a Keuffel and Esser direct-reading spectrophotometer. The re-

flection measurements thus obtained were plotted in the form of a spectrophotometric curve and also converted into terms of the red, green, and violet excitation values. From these values the dominant wave length and percentage of purity were read from a copy of the chart as published in the report of the committee of the Optical Society of America (**56**) on colorimetry for 1920–21. The percentage of relative brightness was also computed so that the colors of fruits are given in numerical expression of the three attributes of color, namely, hue, saturation, and brilliance.

In describing the color stimulus in terms of the psychological sensation which it produces, all of these attributes of color must be considered. The Committee of the Optical Society of America (**56**) suggests the following definitions: "Brilliance is that attribute of any color in respect of which it may be classed as equivalent to some member of a series of grays ranging between black and white." Brilliance is expressed as percentage of relative brightness. The percentage of brightness of a color defines that proportion of the total amount of white light falling upon it that the color is capable of reflecting or transmitting. All colors except black exhibit brilliance. The brightness of absolute black is zero, that of pure white 100 per cent.

"Hue is that attribute of certain colors in respect of which they differ characteristically from the gray of the same brilliance and which permits them to be classed as reddish, yellowish, greenish, or bluish." Hue is expressed as dominant wave length.

"Saturation is that attribute of all colors possessing a hue, which determines their degree of difference from a gray of the same brilliance." Saturation is expressed as percentage of purity. All colors which exhibit a hue must also exhibit a saturation. Grays have zero percentage purity. Saturation determines the degree in which a color possesses hue; thus, the percentage purity defines how red or how green, or how yellow a color is. Purity and chroma are synonymous terms.

For example, spectrophotometric reflection measurements show the color of a ripe tomato as having a brightness of 11.8 per cent. a dominant wave length of 609 mμ, and a purity of 24.5 per cent. The spectrophotometric curve is presented in figure 1; the dominant wave length shown by the vertical line falls in the orange-red region of the spectrum, and the observer pictures an attractive red fruit. A red and a pink may have the same dominant wave length but will differ in percentage of purity; pink, being an unsaturated red, will have a lower percentage of purtiy. A pure spectral red has a purity of 100 per cent. On the other hand, the red will have a lower percentage of brightness owing to the lesser proportion of white in it. Where the curve is an approximate straight line parallel to the abscissa, the color is said to be gray. The horizontal line of zero relative energy will represent black, whereas the line of 100 relative energy will represent white.

Curve 2 is a spectral reflection curve of a Marglobe tomato ripened at 32° C. and which appears to the eye as a brilliant yellow. In numerical terms the color of this fruit would be described as possessing a brightness of 33.5 per cent., a dominant wave length of 581.5 mμ, and a purity of 59 per cent. Curve 3 is plotted from reflection measurements taken from a typical Marglobe tomato picked in the so-called "mature green" stage. The color of this fruit is described as having a brightness of 32.9 per cent., a dominant wave length of 570 mμ, and a purity of 53 per cent. Such a color indicates a yellowish green in which the green components of chlorophyll to a large extent mask the yellow ones.

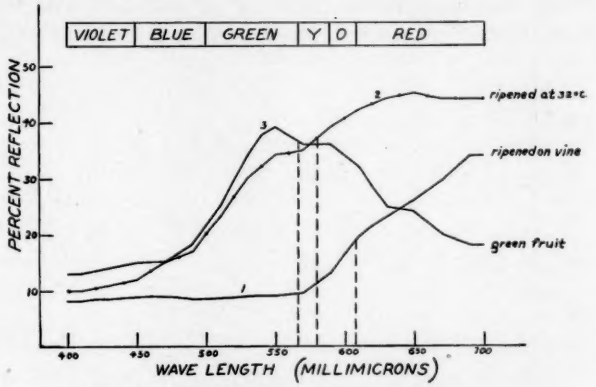

FIG. 1. Color analysis of Marglobe tomatoes.

This method of color analysis was chosen because the spectrophotometer is an instrument of precision by means of which the attributes of color can be measured accurately. As previously pointed out, the eye is not an analytical instrument, and, with advance in color measurement, spectrophotometers will undoubtedly become more readily available in the future.

Effect of temperature

EFFECT OF TEMPERATURE UPON DEVELOPMENT OF COLOR IN TOMATO

In an attempt to ripen quickly some tomato fruits which were gathered green to avoid losing the fruits from frost DUGGAR (**18**) made the discovery that those fruits ripened at a temperature of 20° C. in the dark for seven days had a much redder color than those stored in an incubator at a temperature of 35° C. for an equal length of time. This observation led to a series of carefully planned and executed experiments on greenhouse-grown Earliana tomatoes. Later similar experiments were conducted on species of *Capsicum* and *Momordica*. In all cases fruits stored at temperatures above 30° C. and

below 37° C. showed very little production of lycopene and were distinctly yellow in color. The same results were obtained when only half-grown fruits were tried and also when the experiments were extended to cover fruits developing on the vine. These facts led DUGGAR to conclude that the optimum temperature range for lycopene formation is narrow and that its optimum coincided with the optimum for growth or perhaps was a few degrees lower. Furthermore, the factors for reddening were not destroyed by a temperature of 37° C., for when such fruits were returned to favorable temperature of 20° C., lycopene development proceeded and in four or five days the yellow fruits became red. EULER, KARRER, KRAUS, and WALKER (**20**) confirmed the observation that temperatures above 30° C. produce tomato fruits which are yellow in color. They suggest that the yellow pigment so induced is not

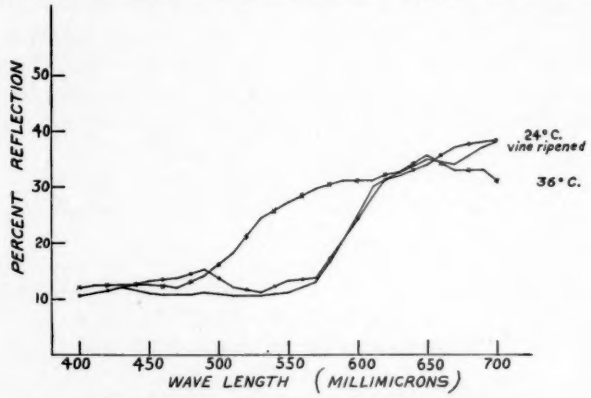

FIG. 2. Effect of temperature (24°, 36° C.).

of a carotenoid nature but is possibly a flavone or flavinol. However, the last named authors make the clear assumption that lycopene formation is an enzymatic process and that lycopene synthesis does not occur at temperatures above 30° C. because of the destruction of the unknown enzyme.

In the experimental work herein reported the author used Bonny Best and Marglobe varieties of tomatoes. The fruits were picked green and selected for uniformity in size and in approximate age from blossoming time. The fruits were then stored for twelve to twenty-four hours at 20° C. and again finally selected for experimental work. The fruits were placed in darkness in constant temperature cases of two and one-quarter cubic feet capacity and the temperatures were held at 24°, 28°, 32°, 36°, and 40° C. (± 1° C.). Reflection measurements were taken with a Keuffel and Esser spectrophotometer at the close of a seven-day period in the case of series 1 and 4, and at the end of a nine-day period in series 2 and 3. Series 2, 3, and 4 served also

as checks in the work on the effect of ethylene, of varying concentration of oxygen, and of light respectively. Moisture was supplied by evaporation from open dishes in each constant temperature case. Reflection measurements were taken from three representative tomatoes in each case. To obtain a flat surface for reflection a section of the pericarp wall 22 mm. in diameter was removed with a sharp cork borer and this section mounted in the 25-mm. cell which accompanies the instrument. Such a section when flattened filled the cell and gave a uniform reflection surface. Measurements were recorded at 20-mμ intervals from 450 to 690 mμ respectively. Since the analytical work requires a reading at 10 mμ intervals from 400 to 700 mμ respectively, assumed readings were interpolated or extrapolated as necessary. However, the sensitivity of the eye below 450 and above 690 mμ is so

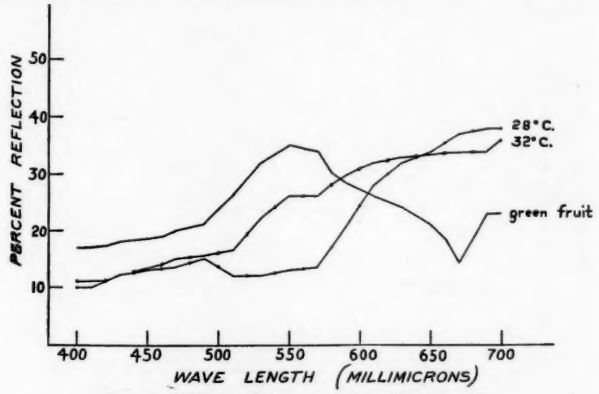

FIG. 3. Effect of temperature (28°, 32° C.).

low that an assumed value is sufficiently accurate. The percentage of reflection was recorded, and the red, green, and violet sensations and the relative luminosity as compared to the luminosity of noon-day sun were calculated with the aid of a special Keuffel and Esser slide rule. From these data the brightness, dominant wave length, and purity of each sample were calculated and are presented in table II.

The most remarkable observation in respect to the effect of temperature (fig. 4) is the great difference in percentage of brightness and percentage of purity of samples ripened at 24° and 28° C. as compared with those ripened at 32° and 36° C. This difference is even more pronounced when dominant wave length is considered. Fruits ripened at 32° and 36° C. show a dominant wave length of from 500 to 588 mμ, appearing bright yellow, while those ripened at 24° and 28° C. show a dominant wave length of from 595.5 to 609.5 mμ and appear a deep orange-red to the eye. Spectral reflection curves are presented in figures 2 and 3.

When the bright yellow fruits ripened at 32° and 36° C. are returned to a temperature 20° C., they rapidly turn orange-red and show a dominant wave length of 592.5 to 597.0 mμ. After four days at 20° C., such fruits are as red as normal fruits ripened on the vine and show a dominant wave length of from 600 to 610 mμ.

A second remarkable effect of temperature of great importance is the fact that both Bonny Best and Marglobe varieties of tomatoes when ripened at 40° C. do not turn yellow but remain green, *i.e.*, chlorophyll decomposition is prevented. Frequently brown spots of various size and shape develop after four or five days and such fruits in which normal metabolic activity is at least seriously checked are quickly attacked by bacteria and other fungi. Fruits ripened at 40° C. for seven days show a dominant wave length of

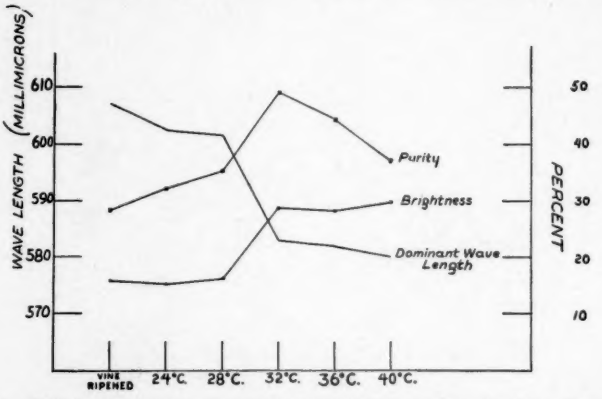

FIG. 4. Effect of temperature on purity, brightness, and dominant wave length.

578.5 mμ, a brightness of only 22.96 per cent., and a purity of 22.5 per cent. and appear a dull green to the eye. This low purity as compared with normal green fruit indicates unsaturation, *i.e.* a gray-green of low brilliance, having a larger proportion of white admixed. When these fruits ripened at 40° C. are transferred to a temperature of 20° C., they do not produce lycopene as do the fruits ripened at 32° or 36° C. but very quickly undergo internal breakdown. Such fruit are particularly subject to attack by *Bacillus carotovorus, Phoma* sp., *Rhizopus nigricans,* and other saprophytic organisms. The normal metabolic activity of such fruits is seriously interfered with, resulting in the more or less complete disorganization of the plasma structure. With this disorganization the complete destruction of the whole cellular mechanism for lycopene formation takes place. This great difference which is produced when the temperature is changed from 36° to 40° C. is remarkable and with other previously mentioned reasons indicates that

TABLE II

EFFECT OF TEMPERATURE UPON PRODUCTION OF COLOR IN TOMATO

SERIES 1 + 4–9 DA., 2 + 3–7 DA.	BRIGHTNESS				DOMINANT WAVE LENGTH				PURITY			
	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 1	SERIES 2	SERIES 3	SERIES 4
Vine ripened fruit	16.33	17.68	16.93	11.80	610.0	605.0	605.0	609.0	25.0	32.0	31.5	24.5
Green fruit	29.91	30.45	26.26	31.21	565.0	570.0	577.5	562.0	35.0	40.0	37.0	40.0
24° C.	16.93	16.74	15.73	11.38	608.0	600.0	600.0	602.5	23.0	30.0	32.5	33.0
28° C.	15.89	17.91	16.06	14.17	609.5	600.0	595.5	604.0	22.0	35.0	46.0	38.5
32° C.	25.36	22.32	30.81	36.83	582.5	588.0	582.0	580.0	45.5	42.5	57.0	51.0
36° C.	25.69	26.02	33.39	28.31	582.0	580.0	583.5	582.5	50.0	32.0	51.2	44.0
40° C.	22.96	33.65	32.74	30.21	578.5	580.0	581.0	579.0	22.5	39.0	42.0	45.0
32° C. + 2 da. 20° C.	19.02	.....	.....	.....	597.0	.....	.....	.....	29.00	.....	.....	.....
32° C. + 4 da. 20° C.	16.63	.....	.....	.....	605.0	.....	.....	.....	30.00	.....	.....	.....
36° C. + 2 da. 20° C.	18.51	.....	.....	.....	592.5	.....	.....	.....	42.00	.....	.....	.....
36° C. + 4 da. 20° C.	17.35	.....	.....	.....	600.0	.....	.....	.....	32.00	.....	.....	.....

lycopene formation is not a simple enzymatic change but, like the decomposition of chlorophyll, is an internal change which can take place only in actively metabolic cells, and emphasizes the physiological inequalities of different temperature ranges.

Effect of temperature upon development of color in watermelon

Both Karrer (**3**) and Zechmeister (**8**) state that lycopene is the red pigment in the watermelon (*Citrullus vulgaris* Schrad.). A series of experiments was planned to determine the effect of temperature upon lycopene production in this fruit. It was found to be impossible to hold green watermelon fruits at temperatures of 24° to 40° C. for more than a few days. Fruits which were removed from the vine were quickly attacked by saprophytic fungi and were reduced to a decayed mass in a relatively short time. One lot of melons, variety Winter Queen, were successfully held at 24°, 28° and 32° C. for fifteen days.

The spectrophotometric data for these fruits, as seen in the accompanying table, show that in fifteen days the fruits have undergone very little change regardless of the temperature at which they were held. This difference in behavior between the watermelon and the tomato led to further attempts to ripen watermelon fruits under temperature control. All other attempts to artificially ripen watermelons off the vine gave negative results because of fungus attack. Removal of fruits with ten to fifteen feet of vine, trial feeding of immature melons with glucose, and several other attempts proved futile. However, it was deemed practical to move temperature equipment to the field. Three fruits of variety Arikara were held at controlled temperature for nineteen days. One fruit was kept in the dark at 18° to 22° C., another was kept at 33° to 37° C. in the light, and a third melon was held in the same higher temperature case but in the dark. The color analyses of such fruits show close agreement, brightness varying from 14.62 to 16.43 per cent., dominant wave length from 592 to 595 mμ, and purity from 44 to 50 per cent. (table III).

These data indicate that perhaps the same mechanism for lycopene formation does not exist in the watermelon as in the tomato. A shift in temperature from 20° to 37° C. did not result in the prevention of lycopene formation as in the case of the tomato,the red pigment production continuing without apparent interruption regardless of the temperature. Either lycopene formation proceeds in a different fashion in the watermelon than in the tomato or else we are dealing with a red pigment other than lycopene. Bilger (**10**) has recently shown that the red pigment from the Japan red pepper differs in chemical composition from lycopene or from the Zechmeister-Cholnoky red pigment from paprika, and that this new pigment is not an isomer of carotene. However, Brown (**12**), working with the red

TABLE III

EFFECT OF TEMPERATURE UPON DEVELOPMENT OF COLOR IN WATERMELON

	BRIGHTNESS	DOMINANT WAVE LENGTH	PURITY
		Variety Winter Queen	
		$m\mu$	
Green fruit	31.45	585.0	16.0
24° C. 15 days	28.34	589.0	16.0
28° C. 15 "	21.06	588.0	23.0
32° C. 15 "	31.27	584.5	17.0
36° C. 15 "	data not available due to decay		
40° C. 15 "	data not available due to decay		
		Variety Arikara (vine ripened)	
18°–22° C. 19 days, dark	16.43	592.0	50.0
33°–37° C. 19 days, light	14.62	595.0	44.0
33°–37° C. 19 days, dark	15.84	595.0	44.0

pigment of the Perfection pimento (*Capsicum annuum*), finds it to be identical with the capsanthin isolated by ZECHMEISTER and CHOLNOKY (**62**) from the Hungarian paprika. Quantitative data in regard to the identification of the red pigment of the watermelon is needed. Data derived from breeding experiments with both the watermelon and tomato indicate that red is invariably dominant to yellow in hybrids. The genetic ratios obtained suggest that a complex group of factors are present and that they must be in subtle balance. Furthermore, certain unknown metabolic factors are at play which are capable of producing changes within the organism. MUNSELL (**45**) has shown that the red fleshy portion of fresh watermelon tissue when fed to rats as a sole source of vitamin A produces a satisfactory unit gain over an eight-week period. This would lead to the belief that either certain yellow carotenoids must be present in the tissue of the watermelon and that the red pigment would seem to be superimposed on the yellow, as is the case in the tomato, or that certain other antiophthalmic-anti-infective substances are present. Both VON EULER and KARRER state that of all the naturally occurring carotenoids only the carotenes have a provitamin A effect. It seems likely, therefore, that such changes in color in the tomato due to temperature, or lack of such changes in the watermelon, as herein reported, are not the result of a single enzymatic factor but that they are the result of a subtle balance of conditions occurring in the actively metabolic, living cells.

Effect of ethylene upon the production of color in the tomato

Observations in regard to the physiological activity of ethylene go back to the early work on the effect of illuminating gas on plants. GIRARDIN (**23**) was the first worker to describe injury to street trees due to illuminating gas

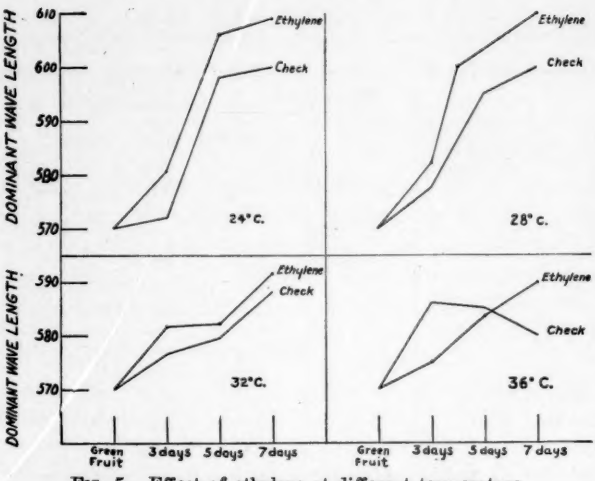

FIG. 5. Effect of ethylene at different temperature.

and KNY (**31**) was the first worker to conduct experimental work on the injurious nature of illuminating gas to maple and linden trees. As early as 1884 MOLISCH (**42**) observed that the linear growth of corn roots was retarded by low concentration of illuminating gas. In 1901 NELJUBOW (**46**) obtained a horizontal curvature of pea and bean seedlings with a concentration of but one part ethylene to one million parts of air. CROCKER and KNIGHT (**15**) determined that the injurious effect of illuminating gas on the flowering of carnations was due to the amount of ethylene which it contained. Later, MOLISCH (**43**), and CROCKER and KNIGHT (**30**) demonstrated that the

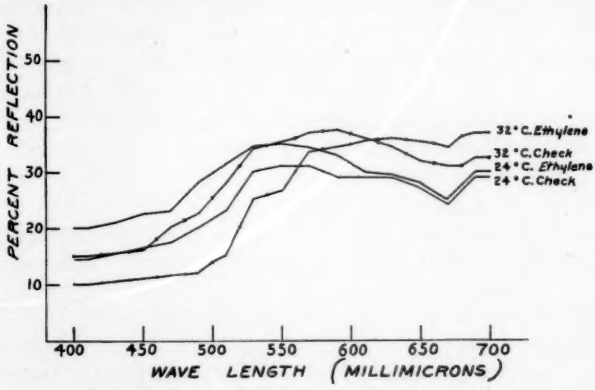

FIG. 6. Effect of ethylene (3 days, 24°, 32° C.).

physiological activity of tobacco smoke was due to the small amount of ethylene present.

SIEVERS and TRUE (**53**) observed the effects of the products of incomplete combustion of kerosene and other substances on the rate of coloring of citrus fruits. DENNY (**16**), trying empirically the substances in pure form which might cause this acceleration of color, was the first investigator to use ethylene for coloration of citrus fruits. By the use of ethylene, self-blanching varieties of celery were blanched by HARVEY (**24**) in six days with a concentration of ethylene of one part to one thousand at a temperature of 18° C. For the dark green varieties a longer time (ten to twelve days) was required. Positive results with ethylene on the ripening of tomatoes and bananas were also obtained by HARVEY (**26**) and successful commercial methods were worked out and established.

Recently ELMER (**19**) discovered that certain volatile substances from several varieties of apples caused an inhibition of growth of potato sprouts. GANE (**22**) was able to identify analytically the active volatile substance as ethylene, the amount produced being very small. NELSON and HARVEY (**47**), using the leaf epinasty method, were also able to show that Golden self-blanching celery produces in normal metabolism some gaseous or volatile compound and that the decomposition of chlorophyll is accomplished by some process similar to that by which celery is commonly blanched artificially by ethylene.

TABLE IV

EFFECT OF ETHYLENE ON PRODUCTION OF COLOR IN TOMATO

	BRIGHTNESS		DOMINANT WAVE LENGTH		PURITY	
	ETHYLENE	CHECK	ETHYLENE	CHECK	ETHYLENE	CHECK
Vine ripened	.....	17.68	.....	605.0	.....	32.0
Green fruit	.....	30.45	.....	570.0	.....	40.0
24° C. 3 days	29.07	32.61	580.5	572.0	39.5	28.0
28° C. 3 "	26.00	32.27	582.0	577.5	43.0	28.5
32° C. 3 "	28.11	33.94	581.5	576.5	58.0	47.0
36° C. 3 "	38.37	21.60	575.0	586.0	40.5	23.5
24° C. 5 days	17.26	16.66	606.0	598.5	29.0	36.5
28° C. 5 "	14.57	16.72	600.0	595.0	36.0	30.5
32° C. 5 "	30.50	34.03	582.0	579.5	47.5	39.0
36° C. 5 "	34.78	28.45	583.5	585.0	45.0	44.0
24° C. 7 days	15.26	16.74	609.0	600.0	31.5	30.0
28° C. 7 "	14.71	17.91	610.0	600.0	32.0	35.0
32° C. 7 "	23.01	22.32	591.5	588.0	44.0	42.5
36° C. 7 "	20.43	26.02	590.0	580.0	40.5	32.0

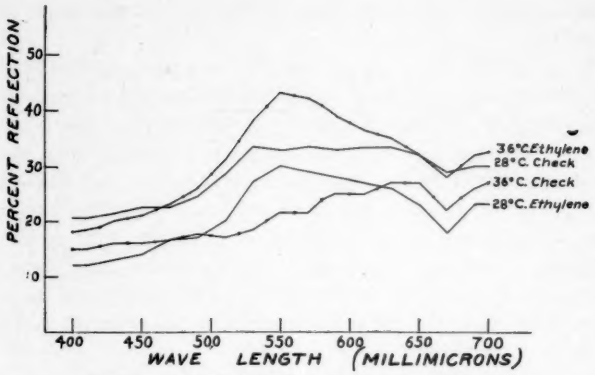

FIG. 7. Effect of ethylene (3 days, 28°, 36° C.).

The production of the red color of the tomato is due to the formation of lycopene within the actively metabolizing cells of the fruit. For this red color to be manifest the chlorophyll must first be decomposed. The data presented in table IV show that, under the influence of ethylene, the decomposition of chlorophyll is hastened and that the production of lycopene quickly follows, providing the temperature is suitable.

Bonny Best tomatoes were used in this work and received ethylene gas at the rate of one part per thousand on three successive days. The fact that ethylene-ripened fruits are redder than untreated ones in the same length of time is clearly demonstrated when dominant wave length is plotted against time, as shown in figure 5. However, it should be clearly stated that lycopene formation does not take place at temperatures of 32° or 36° C. whether

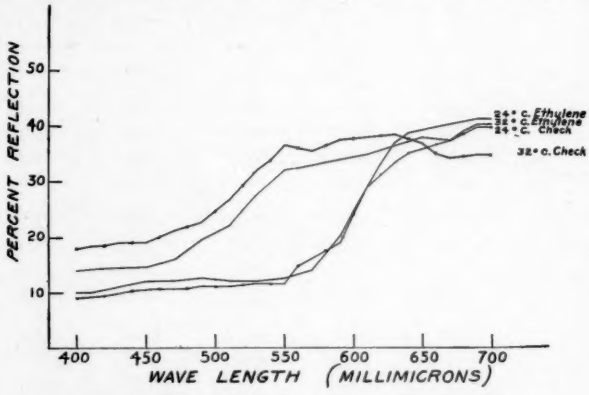

FIG. 8. Effect of ethylene (5 days, 24°, 32° C.).

treated with ethylene or not. Undoubtedly, some of the commercial failures to hasten the reddening of tomatoes by the use of ethylene are due to the fact that lycopene formation has such a narrow temperature range. Fruits ripened at 32° C. or above possess a dominant wave length of 575 to 591.5 mμ and a relatively high purity. Such fruits appear a bright yellow to the eye. When these yellow fruits are returned to a temperature of 20° C. to 28° C., lycopene formation occurs rapidly. However, such yellow fruits can be maintained in the yellow condition for from two to three weeks by storage at a temperature of 2° C. Evidently lycopene formation does not necessarily follow chlorophyll decomposition, and if lycopene is formed from colorless chlorophyll decomposition products, its synthesis involves a mechan-

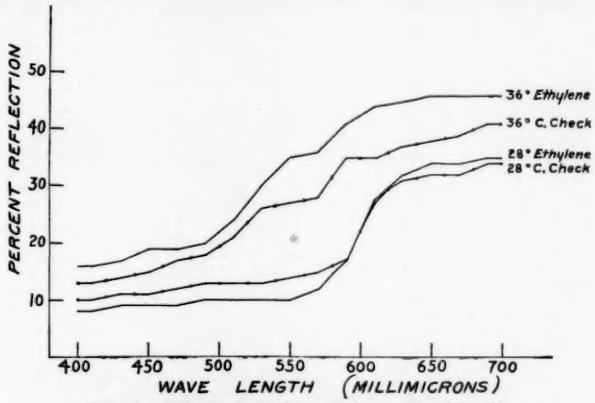

FIG. 9. Effect of ethylene (5 days, 28°, 36° C.).

ism different from the mechanism responsible for chlorophyll decomposition. The data in table IV (plotted in fig. 5) also show that ethylene hastens lycopene formation as well as chlorophyll decomposition. The ethylene-treated fruits possess a dominant wave length of 600 to 606 mμ at the end of a five-day-ripening period whereas the dominant wave length of those fruits ripened without ethylene varies from 595 to 598.5 mμ and appears more orange-red to the eye than those of the ethylene group (figs. 8, 9). Likewise, at the end of a seven-day period the ethylene-ripened fruits show a higher dominant wave length and thus would appear redder to the eye (figs. 10, 11).

Little is actually known in regard to the mode of action of ethylene in the living cell. A review of the literature shows that ethylene is exceedingly active and effects a great diversity of reactions. It is capable of markedly modifying the growth rate of meristematic tissue; of producing intumescences in many different plant tissues; of bringing about premature abscission of leaves; of producing specific epinastic response of petioles at a

minimum concentration of but one part in ten millions; of greatly increasing the oxidation rate in tissues exposed to it even in very low concentrations; of giving increase in soluble sugars and amino acids, at the expense of the insoluble carbohydrates, proteins, and fats; and of hastening the process of chlorophyll decomposition. Data presented above adds weight to the latter statement and, in addition, show that lycopene formation is materially hastened by ethylene, provided a suitable temperature is maintained.

Effect of varying concentrations of oxygen upon production of color in tomato

In agreement with EULER (2) and KARRER (20), LUBIMENKO (36, 37) holds the view that the process of ripening in the tomato is an enzymatic

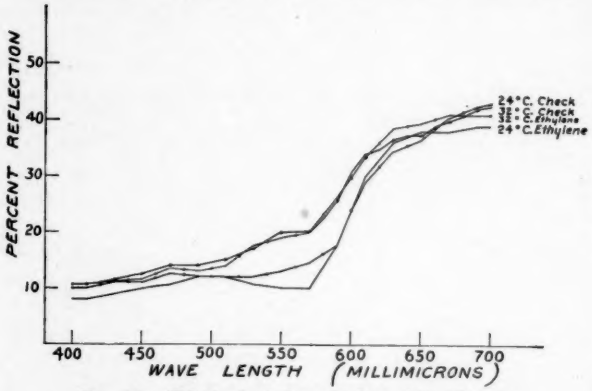

FIG. 10. Effect of ethylene (7 days, 24°, 32° C.).

one. However, he maintains that three separate processes take place in ripening. In the first period when the synthetic reaction is not crowded by oxidation, the accumulation of chlorophyll takes place. Later, oxidation processes predominate, whereby the chlorophyll together with the yellow components are destroyed and transformed into colorless unknown materials. His third postulate has to do with the color-producing activity of the plastid and the accumulation of a red carotenoid pigment within the cells. Whether or not these oxidation processes represent a part of what actually takes place in the cell remains to be seen. Preliminary work on ripening tomatoes in oxygen and other gases indicated that lycopene formation occurs only under conditions favorable for normal metabolism. Green tomatoes placed under pure carbon dioxide and nitrogen gases undergo no development of lycopene regardless of the temperature at which they are held. After five to seven days, such fruits undergo very serious internal breakdown, followed by rapid putrefaction. When the oxygen supply is

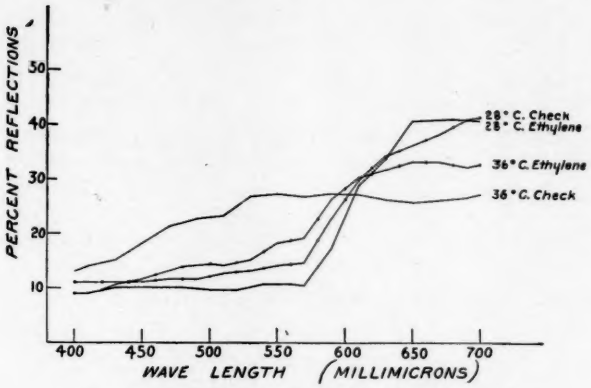

FIG. 11. Effect of ethylene (7 days, 28°, 36° C.).

inadequate, the formation of lycopene does not take place even under favorable temperature conditions. However, chlorophyll decomposition goes on uninterruptedly at low concentrations of oxygen, and at temperatures of 24° and 28° C., as well as at 32° and 36° C., yellow tomatoes are produced (figs. 12–16). Table V shows the dominant wave length of such yellow fruits to be 581.5, 578, 580.5, and 585 mμ at 24°, 28°, 32°, and 36° C. respectively.

Fruits ripened at low and high oxygen concentrations respectively were placed in the constant-temperature cases previously mentioned but within desiccators of approximately three and one-half liters capacity, four fruits being placed in each. Low oxygen concentrations were maintained by keeping the fruits in closed desiccators, which were ventilated with a stream of air for five minutes each twenty-four hours. High oxygen concentration was

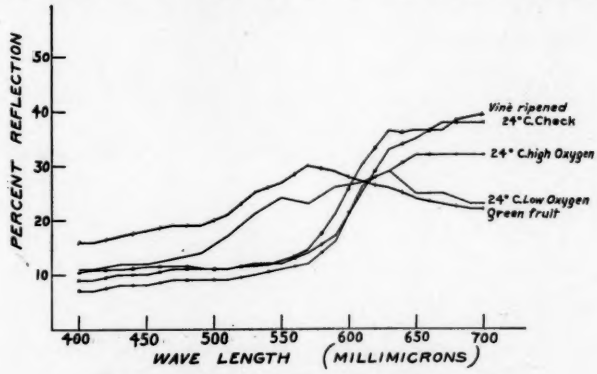

FIG. 12. Effect of oxygen concentration, 24° C.

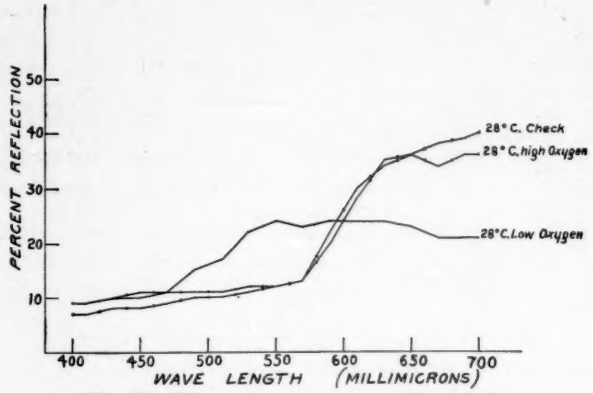

FIG. 13. Effect of oxygen concentration, 28° C.

maintained by slow, constant bubbling of a mixture of 50 per cent. air and 50 per cent. oxygen through the desiccators, the mixture being prepared in and liberated to the desiccators from individual gasometers.

Yellow fruits which were produced under low concentrations of oxygen reddened when returned to the air at 20° C. for four or five days, as also did the yellow fruits which were ripened at 32° and 36° C. in the high oxygen chambers. The close agreement of the dominant wave lengths of fruits ripened at 32° C. is remarkable, the fruits ripened in a low concentration of oxygen having a dominate wave length of 580.5 mμ, the fruits ripened at a high concentration of oxygen having a dominant wave length of 579.5 mμ, and the fruits ripened in air, not being confined to desiccators, having a dominant wave length of 582.0 mμ (fig. 14). The same is true of

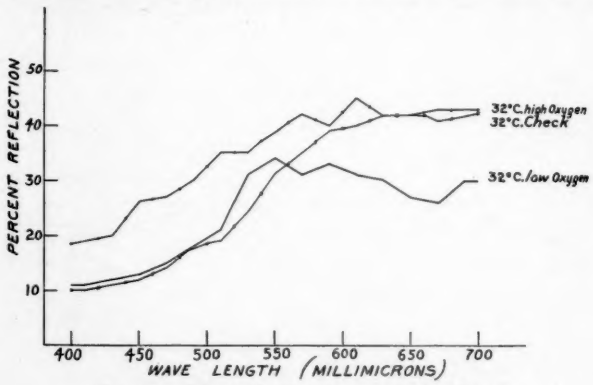

FIG. 14. Effect of oxygen concentration, 32° C.

TABLE V

EFFECT OF VARYING CONCENTRATION OF OXYGEN ON PRODUCTION OF COLOR IN TOMATO

	DOMINANT			BRIGHTNESS WAVE LENGTH			PURITY		
	LOW 0_2	HIGH 0_2	CHECK	LOW 0_2	HIGH 0_2	CHECK	LOW 0_2	HIGH 0_2	CHECK
Vine ripened	.....	.....	16.93	.....	.....	605.0	.....	.....	31.5
Green fruit	.....	.....	26.26	.....	.....	577.5	.....	.....	37.0
24° C. 7 da.	22.84	14.18	15.73	581.5	598.5	600.0	44.0	40.0	32.5
28° C. 7 da.	22.06	16.76	16.06	578.0	601.0	595.5	49.0	35.0	46.0
32° C. 7 da.	27.79	38.68	30.81	580.5	579.5	582.0	52.0	35.0	57.0
36° C. 7 da.	23.55	21.10	33.39	585.0	583.5	583.5	54.0	43.0	51.5

those fruits held at 36° C. having a dominant wave length of 585.0, 583.5, and 583.5 in low oxygen, high oxygen, and air respectively (fig. 15). This indicates that chlorophyll decomposition goes on at both low and high concentrations of oxygen but that the formation of lycopene is prevented when the oxygen supply is decreased, as in the case of fruit ripened under the partial anaerobic conditions of the experiment. That temperatures above 30° C. inhibit the production of lycopene is again apparent, which together with the before mentioned observation that suitable quantities of oxygen must be present for lycopene formation to take place indicates that beyond all reasonable doubt, the production of lycopene is a process which can take place only when the cells are actively metabolizing and that it seems to be more than a simple enzymatic change.

Effect of light upon the production of color in the tomato

The discovery by HARVEY (**27**) of the importance of light as a factor in the decomposition of the carotenoids present in butter and in the peel of

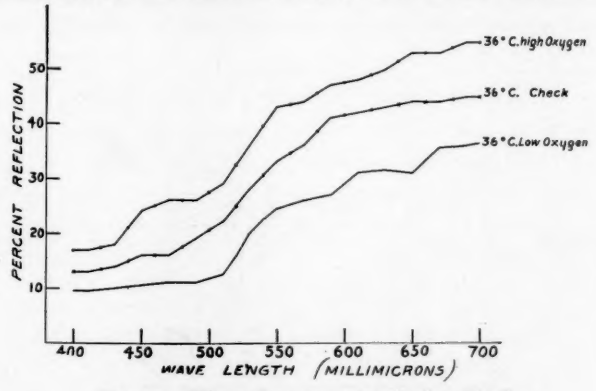

FIG. 15. Effect of oxygen concentration, 36° C.

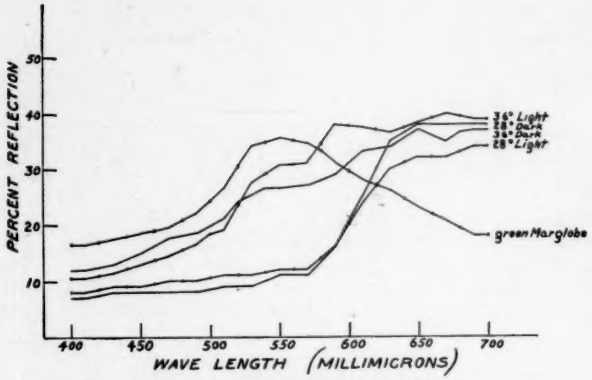

FIG. 16. Effect of light, 28°, 36° C.

orange fruits in the presence of benzaldehyde led to a series of experiments in which Marglobe tomatoes picked in the full-grown, green stage were ripened at temperatures of 24°, 28°, 32°, and 36° C. in total darkness and illuminated for twenty-four hours daily. The light source was a 1000-watt Mazda bulb giving an illumination of 1540 to 1620 foot-candles as measured by the Weston photronic cell, model 603. That chlorophyll decomposition is hastened by light is not denied but from the data presented in table VI lycopene formation is shown to proceed equally well in either light or darkness, provided the fruits are maintained in the presence of air and at a suitable temperature, namely, 20° to 28° C. (figs. 16, 17). Also, light may affect the yellow carotenoid constituents of the tomato, but the data indicate that temperature is the limiting factor in lycopene production. This also seems to hold true for the decomposition of other carotenoids. It was considered that light absorbed by chlorophyll and by carotenoid pigments might activate the decomposition of these pigments owing to the energy absorption,

TABLE VI

EFFECT OF LIGHT ON PRODUCTION OF COLOR IN TOMATO

	BRIGHTNESS		DOMINANT WAVE LENGTH		PURITY	
	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK
Vine ripened fruit	11.80	.....	609.0	.....	24.5	.....
Green fruit	31.21	.....	562.0	.....	40.0	.....
24° C. 9 da.	11.68	11.38	604.0	602.5	30.0	33.0
28° C. 9 da.	14.70	14.17	599.0	604.0	34.0	38.5
32° C. 9 da.	33.30	36.83	581.0	580.0	48.0	51.0
36° C. 9 da.	30.27	28.31	581.5	582.5	54.0	44.0

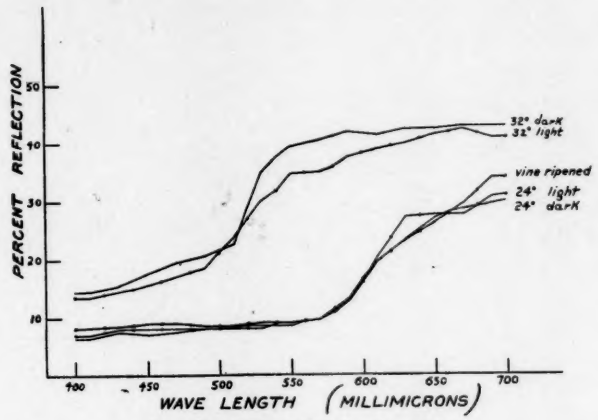

FIG. 17. Effect of light, 24°, 32° C.

but the data here presented indicate that this physical factor does not accelerate chlorophyll decomposition or the removal of carotenoids from the fruit in an appreciable amount. The data of HARVEY were obtained in the presence of benzaldehyde which may form active peroxides on light exposure of wave lengths which are absorbed by the carotenoids, and thus lead to their rapid decomposition. The tomato evidently does not produce such aldehydes as appear in the seeds of certain rosaceous plants. However, further explanation must await further progress in the explanation of certain internal changes which take place within the plasma complex of the living plastid.

Summary

1. The optimum temperature for lycopene formation in the tomato was found to be 24° C. Lycopene is not formed at temperatures above 30° C. When tomatoes are ripened at 32° to 38° C., bright yellow fruits are produced. When such yellow fruits are returned to 20° to 24° C., lycopene is developed normally.

2. Chlorophyll decomposition in tomato fruits is prevented by a temperature of 40° C. or higher. Such fruits remain green and neither produce lycopene when returned to a temperature of 20° to 24° C. nor the yellow pigments when returned to 32° C.

3. In the case of the watermelon, a shift in temperature from 20° to 37° C. did not check the production of the red pigment, indicating that the same mechanism for lycopene formation does not exist in the watermelon as in the tomato. It seems likely that color changes in the tomato due to temperature, or lack of such changes in the watermelon, are not the result

of a single enzymatic factor but are the result of a subtle balance of conditions occurring in the actively metabolic cells.

4. Ethylene hastens lycopene formation as well as chlorophyll decomposition in the tomato, provided a suitable temperature is maintained.

5. Lycopene formation is prevented when the oxygen supply is decreased, chlorophyll decomposition going on uninterruptedly within the 24° to 36° C. temperature range.

6. Chlorophyll decomposition is hastened by light, but lycopene formation proceeds equally well in either light or darkness provided the fruits are maintained in the presence of air and at a suitable temperature.

The work here reported was performed in the Laboratory of Plant Physiology, University of Minnesota, and the writer wishes to acknowledge the assistance and encouragement of Professor R. B. HARVEY. Thanks are also due Professor R. A. GORTNER, University of Minnesota, for loan of the spectrophotometer, and to the Keuffel and Esser Co., Hoboken, New Jersey, for their helpful suggestions.

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EFFECTS OF IONIZED AIR AND OZONE ON PLANTS

CLYDE HOMAN

(WITH FIVE FIGURES)

Introduction

Beginning as far back as the latter part of the eighteenth century, numerous investigators have subjected plants to the influence of electrical fields of widely different potentials and frequencies, using various methods of application, often with the idea of obtaining some growth-stimulating effect. Adequate controls were generally not maintained, nor were the conditions used possible of duplication owing to lack of measurements of environmental factors. Although results were reported varying from greatly increased yields to disastrous effects, later extensive and more carefully controlled experiments (**2, 6**) tend to disprove any effect on growth because of potential gradient alone.

About 1914 it was suggested that such effects on growth, as were observed when using electrical discharges, were not due to the effect of a potential difference on physiological functions of the plant, but merely to secondary factors resulting from the electrical discharge, *e.g.*, formation of ions, ozone, active oxides of nitrogen, etc., (**5, 10, 21**). As far as can be found from the literature, there have been no experiments in which the mentioned factors have been isolated. There has been no work done in which these factors have been measured by any reliable method, either when attempts were made to isolate them, or under the conditions of a high tension discharge.

Workers who have used ultraviolet rays, x-rays, and radioactive materials, as well as electrical discharges, have employed agents that ionize the air and produce ozone to a greater or lesser extent; and to them a knowledge of the effects of these factors is of interest.

Results of previous investigators

The first discussion of ionized air in this connection occurred in such papers as those of BRESLAUER (**1**) and JØRGENSEN (**10**). These papers refer to simple experiments with overhead wires, with the assumption that the air is more or less ionized by such discharges, but making no mention of measuring the ionization, if any, that may have occurred in the vicinity of the plants.

More recently experimental methods have been developed to determine whether ionized air has a direct influence upon plant growth. However, an analysis from the physicists' point of view of the previous experimental work with ionized air discloses a number of errors in method and interpretation sufficiently serious to invalidate most of the conclusions reached. Some of the most obvious of these errors may be pointed out.

Using polonium on copper foil as an ionizing agent for air, which was then passed over the plant material, MIDDLETON (**16**), WHIMSTER (**29**), VAN ASPEREN DE BOER (**28**) and PORTSMOUTH (**19**), obtained contradictory results. MIDDLETON found an increase in respiration varying from 5.58 ± 2.57 to 29.11 ± 5.62 per cent. PORTSMOUTH, attempting to duplicate MIDDLETON'S results with barley seedlings, got no significant increase in one experiment, and only 1.5 per cent. in another. Again, WHIMSTER found an increase as high as 85.7 ± 7.1 per cent. in respiration of *Pelargonium* leaves, while PORTSMOUTH with a similar experimental setup obtained no sign of the increase found by WHIMSTER. Likewise VAN ASPEREN DE BOER found no effect of ionized air on the respiration of fungi.

These men assumed that only alpha particles are emitted by polonium. However, RUSSELL and CHADWICK (**20**) found both a gamma and a beta radiation, the gamma being three times as strong when the polonium was deposited on copper as when deposited on aluminum. Therefore the results of the experiments given above may have been influenced by these gamma and beta radiations.

In these experiments, moreover, the number of ions per cc. of air was calculated from the saturation current and the volume of air between two plates placed in the position occupied by the plant material used. Of course the field would extend beyond the plates, and ions would be drawn from a considerable distance; but it is of much greater importance that the use of such high potentials as 947 volts across plates 2–5 cm. apart resulted in the electric field simply drawing the ions to the plates as fast as they were formed (the polonium being at a distance of only 4–5 cm.). What they really calculated was the rate of production of ions. The *number of ions per cc.* would not be measured by this procedure. The amount of ionization at a given moment would depend partly on the rate of recombination of ions, which would greatly lessen the number, as would also diffusion of ions and the movement of air. The voltage used was so high that no other evidence is needed that the ions were drawn to the plates as fast as formed, the mobilities of the ions being about 90 cm. per second.

SPOEHR (**23**) drew air, supposedly ionized by sunlight, from outdoors over wheat seedlings and onions, and measured the CO_2 output during the day and night. He found that when the air was not de-ionized by passing through an electric field, the ratio of CO_2 output per hour by day to that by night was 1.15 for onions and 1.042 and 1.091 in two studies of wheat seedlings. In two experiments, in which he de-ionized the air drawn in before passing it over the plants, the ratios were 1.010 and 1.015. This would indicate that air exposed to daylight with its slightly higher degree of ionization caused slightly increased respiration, provided no other changes had also been brought about. It would seem, however, that drawing the air

through 45 feet of glass tubing in a thermostat would allow sufficient time for considerable recombination to take effect, possibly nullifying the higher ionization due to the sun's rays. There was no measurement of the degree of ionization of the air, reliance being put on the work of other investigators, which shows, in general, that there is a higher ionization of the air in sunlight than in darkness. There has evidently been no confirmation of this work.

HENRICI (**7, 8**) noted in the course of her experiments that the assimilation of alpine plants was much more active before a thunderstorm in spite of the low intensity of the light. She ionized the air artificially by the use of thorium oxide, and protected the plants by aluminum screens from any alpha radiation. She found that for lowland plants ionization favors assimilation in weak light, has no effect in medium light, and retards it in strong light; for alpine plants ionization increases assimilation in weak and medium light, and has no effect in strong light. She claims that in suitable conditions of light, ionized air increases photosynthesis; in some cases she got as high a ratio as 3–4:1. Her results, however, were not based on the averages of a sufficiently large number of experiments for calculating the probable error; hence it is not possible to estimate their value. Calcium hydroxide solution in Pettenkoffer tubes was used for absorption of CO_2 and it does not seem that the small differences in CO_2 found could have been very accurately measured. She has not continued with this work as far as can be determined from the literature, and there has been no confirmation from other sources. No measurements as to degree of ionization produced were made. Methods used to control temperature, etc., were not clearly described. Also, thorium oxide is a very weak ionizing agent, and aluminum sheets would not stop gamma rays.

LIPPERHEIDE (**14**), using sparks as a source of ionized air, presented the results and conclusions of a number of experiments mainly on the supposed effect of ionized air under the conditions in which he produced them. The conditions of his experiments are briefly as follows: Potted bean plants, *Phaseolus vulgaris,* were grown in garden soil in cases with glass tops. The condition of the air in the treated case was modified by blowing air through two metal combs placed opposite each other. Sparks were passed between the points by connection with an induction coil and other necessary electrical apparatus. Though he does not state the current or sparking distance used, the coil had a maximum capacity of 18 amp. and would give a spark 20 cm. long.

Only a relative idea of the degree of ionization in the treated air as compared to the control was obtained. This was determined by the time it took to discharge a metalfoil-leaf electrometer sufficiently to cause the leaf to move five divisions on the scale. Roughly, the degree of ionization would

be inversely proportional to the time elapsed. The peculiar thing about his figures is that they show great variability in the time required for this degree of discharge of the electrometer. In the control case the time varied from 1 minute and 40 seconds, to 4 minutes, most of the readings being about 4 minutes. In the treated air the variation was from 7 to 50 seconds, mostly around 10 seconds. Thus, the average degree of ionization of the treated air was roughly 24 times that of the control. This gives an ionization of only 24,000 per cc., if the ionization of the control air is assumed to be 1000 per cc. This is much lower than has been obtained by the writer and others referred to, and indicates that the sparks used were not of great number and the field strength comparatively weak. This probably accounts for his being able to grow plants under the conditions employed, for with a sufficiently weak field and sparks the quantity of ozone would be kept below toxic concentration limits. However, he must have been near the border line, for he states: "Die Schädigung durch Ozon war bei manchen Versuchen so gross, dass oft ganze Versuche abgebrochen werden mussten." His results show, as summarized also by KOERNICKE (**13**), that 35 control plants had a leaf surface of 741.37 sq. cm. while 35 treated plants had 1,431.32 sq. cm.; and 31 control plants had a dry weight of 19.057 gm. while 31 treated plants had a dry weight of 33.655 gm. Control plants transpired, during five days, 1299 gm. of water, and the treated plants 1554 gm. Also, during three weeks, when the plants were grown in a nutrient solution, the intake of salts was 7 mg. higher per treated plant than for the controls. Other experiments showed that when the plants were placed in a cellar, a three-peaked curve of leaf motion of small amplitude was obtained, which became a normal two-peaked one of larger amplitude again after "ionization." With stronger ionization, stronger movements were obtained, and with strong ionization the leaves rose. He claims that plants in ionized air remained green in the dark for a longer period; and that when grown in "ionized" air they were darker green and often had a fourth leaflet. This latter observation was emphasized and photographs recorded. This emphasis seems unreasonable. It so happened in the writer's work that this fourth leaflet appeared frequently, but more often in the untreated plants than in the treated.

The above results alone are interesting, but the following criticisms make them untenable:

(1) At all times LIPPERHEIDE used both ionized air and ozone, with possibly small amounts of nitrogen oxides, the amount of O_3 produced depending on humidity, potential used, etc. Up to the time his work was done there had been nothing to show that O_3 in small dosages might not be beneficial. Therefore, assuming all other conditions constant, one would not know to which to attribute the effect. One could possibly assume that when the concentration of O_3 passed a certain point the plants were harmed. This

evidently often occurred. Also, he gives no data on point distances, current, or potential used.

(2) Evidently there was no method of controlling air flow. It seems that all that was done to the control case was to open the door while he was blowing the air past the discharge into the treated chamber, "Dadurch wurde es vermieden, dass den Versuchpflanzen mehr frische Luft zugeführt als den Kontrollen."

(3) How light was controlled is not discussed. It seems it would be very difficult to place two cases in a greenhouse so that the light penetrating the cases was at all times equal.

(4) Where soil was used, no mention is made of how soil moisture was controlled.

(5) Definite mention, however, is made that the humidity varied considerably at times when the readings given were made, there being variations of 25 to 30 per cent. Also some differences are given for temperature. The blower connected with the treated case was evidently only in operation when a discharge was going on between the points. During the remainder of the time, the door of the control case was opened an amount supposed to approximately equalize the effect of the ventilator of the treated case. There is nothing in the description of the construction and setup of the cases which would show that a significant difference in humidity might not have existed most of the time. The writer's work with the same plants indicates that *Phaseolus vulgaris* is particularly susceptible in its growth and size of leaves to moisture conditions.

(6) The work may be criticized because of the small number of plants for which data are given; also, it is not clear on what basis the plants thought to be injured by ozone were eliminated.

(7) From the diagram shown in his article, it appears that the ultraviolet light from the sparks might reach the plants, perhaps having some effect.

In all other experiments listed in the bibliography, no measurements were made, as for instance in the numerous experiments of NEHRU (**17**) in which a variety of sources of supposed electrical energizing were used and seemed in almost all instances to produce stimulating effects.

Lack of controlled conditions as to light, temperature, air flow, etc., are general, as is also the use of a limited number of plants, or the taking of readings of respiration over very short periods, or other similar procedures which would tend to emphasize idiosyncrasies of individuals or of small groups, or differences due to time of day and other local factors. An example of this is the case in which NEHRU (**17**) after uprooting 5 plants, treated them with an electric spark and used only one plant for a control.

Then there exists in some cases questionable accuracy of measurements by absorption or colorimetric methods of the small amounts of CO_2 involved in small differences in respiration.

Available information on effects of ozone on plants

The earliest mention of studies of the effects of ozone on plants was found in a report by HABER (5) of the Kaiser Wilhelm Institute who presented his results at a special meeting. This work has remained unpublished, but the following quotation is taken from the above report:

"He gave the results of his investigations with others of the assimilation of a leaf of cherry laurel in air filled with carbonic acid. It was found that the electric field, either continuous or alternating, produced no change in assimilation unless a glow discharge was obtained. The reaction produced by the glow discharge injured assimilation. The admixture of ozone or oxide of nitrogen with air had the same effect."

The information that this work remained unpublished is taken from STERN (24).

KNIGHT and PRIESTLY (11) passed air through a jar in which an electrical discharge was taking place and thence into a jar containing seedlings. They found the chlorophyll in the upper portions of the seedlings had been bleached. They made no measurements of the products of the discharge but concluded there was no effect on respiration. The bleaching, as will be shown later, was, no doubt, due to a fairly high concentration of ozone.

LIPPERHEIDE (14), in a short paragraph on the influence of ozone on leaf movements of *Phaseolus*, states that the leaves go into the "sleep position" when subjected to a moderate amount of ozone. He finds that the leaves are very sensitive to ozone. If ozone was present for over five minutes, the plants were injured, and after two days were speckled brown, and parts were dead. He adds that ultraviolet rays also cause a downward movement of the leaves and attributes this to ozone arising from the action of ultraviolet radiations on air. No analysis of the gas used was made.

According to authoritative opinion (9), ozone in sufficient concentrations to kill bacteria will kill animals. Apparently, beneficial effects of ozone are due to its odor, which gives relief to the olfactory sense from the tedium resulting from constant inhalation of air at constant humidity and temperature. Also, beneficial effects in diseases of the mucous membranes, if any, are the result of blistering action which may cause increased blood supply.

TAKAMIYA (25), in repeated experiments, has shown that ergosterol exposed to ozone in proper concentration gives a substance having the action of vitamin D, as it also does in ultraviolet light. Thus there might be some beneficial reaction with plant sterols, although work reported in this paper does not indicate it.

Ozone is not found to any extent in lower altitudes due to its decomposition by moisture. In the upper stratosphere, measurements have shown (**15**) that there is an equivalent of a layer about 4.2 cm. thick. MELLOR (**15**) emphasizes the importance of this by quoting B. MOORE as follows:

"As the light from the sun first strikes the earth's atmosphere it is full of ultra-violet light and violet light. Such light rays as these, if they reached the earth at the present moment, would annihilate us; they would simply mean death to the world below. The first thing that happens to prevent this is that ozone is developed, and by absorption this ultra-violet light is shut out."

In regard to the possible production of ozone by plants, URSPRUNG and GOEKEL (**27**) obtained an effect on a photographic plate placed in a closed chamber with plants. As their electrometer showed no change, they concluded that the effect on the plate was due to ozone or other gaseous products and not to ultraviolet radiations which would ionize the air. Results that seem to confirm this idea were given by COUPIN (**3**). A concentrated solution of KI in litmus paper was placed in a chamber with plants and also in a control chamber, and exposure made to diffuse light. The litmus turned blue only in the plant chamber. Evidently some oxidizing agent, perhaps ozone, was formed by the plants which liberated KOH.

Plan of experiments

From the summary just presented, the writer concludes that little can be definitely said, except that O_3 at certain high concentrations was undoubtedly toxic. The possibility remained that ionization alone might have been stimulating, or that ozone in proper concentration might be beneficial to growth. It was, therefore, decided to carry out the following plan:

(1) Determine a method of growing a number of plants at a time under exactly controlled conditions so that it would be possible to change only the gaseous environment of the plants.

(2) Find the best method of producing a high concentration of gaseous ions uncontaminated by ozone.

(3) Devise a method of producing ozone in various desired concentrations without nitric acid anhydride (N_2O_5) being present in any detectable amount.

(4) Grow plants (*Phaseolus vulgaris*) for a sufficient length of time (seedling to blossoming) so that any effect on growth would become definitely evident.

(5) Grow plants in different concentrations of ozone, to determine what concentration is toxic, the specific effects produced, and whether different small concentrations applied either constantly or intermittently are stimulating.

Apparatus and methods

Growth chambers

The plants were grown in experimental cases of the design and dimensions illustrated in figures 1 and 2. The top is set in a trough completely surrounding the sand pan and may be lifted off. This trough is filled with water or other suitable liquid, which acts as an air seal. The glass sides leave the plants visible, while the easy removal of the glass case from over the plants makes it convenient to get at them. To assure complete drainage, the bottom of the sand pan was slightly funnelled.

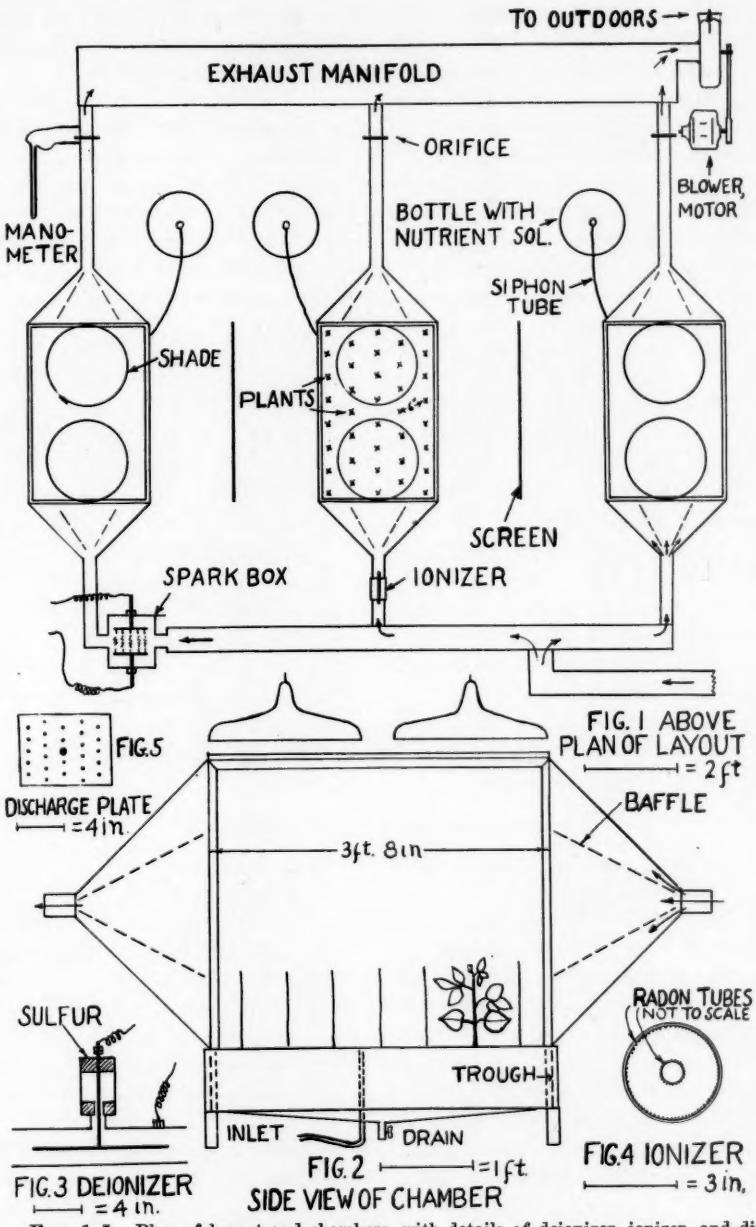
Baffles are soldered into the funnel ends so that air entering and leaving the case is spread out evenly through the interior and does not pass only through the center region. The baffles and funnels are painted dull black to avoid reflection of light. Around the top of the case is fastened a band so that the top glass may be covered with a layer of water.

Three cases were placed in parallel between a common outlet and inlet. One case served as a control for the other two, thus reducing by half the time which would be needed if only two cases were used. Air was drawn through the cases by an exhaust centrifugal blower. Lighting was accomplished by placing incandescent bulbs with reflectors over the cases. The gaseous products were introduced at the inlets.

Control of air flow

In order to be able to maintain equal air conditions in the cases, it was imperative that the air flow be accurately measured and regulated. The common exhaust manifold was 15 inches in diameter so that there would be no drop in pressure with distance from the outlet.

Measuring and regulating air flow was accomplished by the method of differential pressure across an orifice plate placed in the line. The principle is based on Bernoulli's theorem (**12**). If a pipe of perfectly true bore is used, and an orifice plate inserted so that a perfectly round hole is accurately centered, and taps then taken at proper distances, the flow through that pipe may be known with an accuracy of 0.5 per cent. Tables have been worked out (**22**), so that, knowing the fluid and its density, the inside pipe diameter, the ratio of the orifice to diameter of the pipe, and the differential pressure across the orifice, the flow may be found. Correction factors, (also obtained from tables) are introduced for temperature, pressure, and humidity. The tables used here were kindly furnished by the Republic Flow Meters Company of Chicago, Illinois. Of course, the accuracy of the determination of the flow depends entirely on the accuracy with which the diameter of pipe and orifice, and the differential pressure reading are obtained. Seamless steel tubing was used which varied only 1 to 2 thousandths of an inch, and sufficient length was used in front of the orifice so that any turbu-



FIGS. 1–5. Plan of layout and chambers, with details of deionizer, ionizer, and discharge plate.

lence in the flow would be smoothed out. The flange, fitting over the pipes and holding the orifice plates, and the orifice plates themselves, fitted together with the orifice exactly centered. The size of the orifice and the speed of the blower were adjusted by trial so that the flow desired could be obtained with a differential pressure of 70–100 mm. of water, which differences could be read within a fraction of 1 per cent. Even greater accuracy than 0.5 per cent. could be obtatined when simply balancing the flow through two or more identical pipes and orifices, and in reading the differential pressure for accurate adjustment. In this work the limit of error was not over 1 per cent. The greatest errors come from inaccuracies in knowing the diameters of the pipe and orifice, which are perpetuated as the square, while those in reading the differential pressure vary only as the square root.

The blower used was of the ordinary centrifugal fan type (Buffalo Forge Co. no. 1) and was driven by a synchronous motor to avoid changes in flow resulting from fluctuations in line voltage, which was found to vary as much as 10 per cent. when an induction motor was used. As the pressure on the exhaust side of all three orifices was the same, the flow was balanced through the cases by simply adjusting the inlets so that the differential pressure across the orifices were identical. Since the sizes of the three cases and pipes leading in and out of them were all the same, very little adjustment was necessary. It was found that a flow of about 30 cu. ft./min. was suitable, as this changed the air about once a minute in the cases, caused only very trifling movement of the leaves, and when ionized air was being used, was sufficiently rapid so that too large a degree of recombination did not take place. Since air entered the cases from a common manifold and passed through rather rapidly, it was not to be expected that the humidity would differ in the different cases. Measurements of the relative humidity in the three cases showed that there was no detectable variation.

Control of light and temperature

Over each case, at a distance of two inches from the glass, were suspended two 1000-watt bulbs with reflectors. These bulbs were tested for equality of illumination. The glass of the tops was $\frac{1}{8}$ in. thick, the sides $\frac{3}{16}$ in. and of a special high-grade crystal type of glass which was uniform in light transmission and thickness. The top glasses were covered with $\frac{3}{8}$ in. of distilled water, maintained at constant level by an overflow and drip source of replenishment. As dust settled to a slight extent in spite of the fact that the experiment was carried on in an air-quiet basement room and that the reflectors largely covered the tops, the glasses were cleaned daily. Without the water on top, and with an air flow of 30 cu. ft./min., the temperature of air leaving the chamber was 2° or 3° C. higher than that entering; while with the water screen, it was only a fraction of a degree higher. The temperature

in all three cases was equal. But the water was used throughout, not particularly to lower the actual temperature of the air in the cases but to eliminate a large proportion of the infrared rays and so to give a better balanced spectrum and also to prevent internal heating of the leaves. It was found that the plants used showed no curvatures in any direction and grew to uniform heights. There was, therefore, little or no error introduced due to one set of plants growing nearer to the lights, which might tend to exaggerate the differences in growth. Light screens were placed opposite the glass sides of the cases to prevent light from one case being reflected into another.

Control of soil conditions

The plants were grown in pure quartz sand, eight inches deep. Constant soil moisture conditions were obtained by flooding once a day with nutrient solution and draining immediately after. This was accomplished by placing a five-gallon bottle of nutrient solution somewhat above the pans and allowing the solution to siphon to the surface of the sand through a $\frac{1}{2}$ in. pipe from the bottom where it poured into a $1\frac{1}{2}$ in. angle iron with holes bored along its sides. In this way, the liquid flowed fairly uniformly throughout the length of the pan into the sand without disturbing it. When the sand was immersed about $\frac{1}{4}$ in. in the liquid, the inflow was shut off and the drain opened. As the sand in all these pans was of the same particle size and of the same packing tendency, the amount of solution held should have been pratically identical in all three cases. That this was true was shown by measurements of the amount entering, and of the excess that could be drained. Thus soil moisture conditions were constant at the start and would be changed only by differences in degree of transpiration and evaporation during the twenty-four-hour period between waterings. However, there was ample moisture in the sand to last several days and tests showed that it took practically the same amount of water to saturate the sand in each of the three pans after twenty-four hours of transpiration and evaporation. Fresh air was also sucked into the sand when the liquid, filling the intergranular space, was drained.

The nutrient solution used was as follows:

A.	50 gm.	$MgSO_4$	in	500	cc.	H_2O
B.	50 "	KH_2PO_4	"	"	"	"
C.	50 "	$Ca(NO_3)_2$	"	"	"	"
	50 "	$CaCl_2$				
D.	4 "	$FeCl_3 \cdot 6H_2O$	"	300	"	"

Add 10 cc. of solutions A, B, and C, and 10 drops of D to four liters of distilled water.

Obtaining plants of similar size and vigor

About 1000 *Phaseolus vulgaris* seeds of uniform size were selected each time and planted in quartz sand. They were found to germinate uniformly and from this number of plants it was not difficult to obtain 99 plants of almost identical appearance at an early stage in the development of the primary leaves (about ten days after planting). Thirty-three were then planted in each pan (fig. 1), six inches apart and four inches from the sides, in the case of the two rows nearest the glass. At this distance there was little crowding and the plants were not interfered with nor shaded by one another. The plants grew very uniformly and no differences could be noted between the outside and inside rows.

Production and measurement of ionized air

Three sources of ionization of the air drawn into the cases were considered: spark discharges, radioactive compounds, and x-rays. It was at first thought that spark discharges would serve best for a constant source of quantity production of ions and since Lipperheide used this method, it was tried first. A spark discharge box (fig. 5) was built. It consisted of two plates each having twenty-five points spaced 1 in. apart. These were capable of being adjusted so that the points of the opposite plates could be placed at any distance from one another up to 8 in. The two plates were connected to the terminals of the secondary of a transformer that could be regulated up to 100,000 volts. When the current was turned on, a lively display of sparks played between the points. Large Leyden jars (3000 m.m.f. capacity) were placed in parallel with the plates to prevent arching. In this way blue-white, crackling, thick, hot sparks were obtained, which type forms the least ozone. Air was then drawn between the points through the sparks into the plant chambers.

Another method of producing spark discharges was to use a Tesla coil discharge (frequency 4,500,000 cycles per second) connecting the two terminals of the secondary connected to the plates. This gave thinner purplish sparks that became a spray of fine thin sparks spreading between opposite points. This changed almost to the appearance of a glow discharge filling the space between the plates when the points were moved farther apart (6 to 8 in.). The thin sparks produced by the Tesla coil when the points were drawn 4 to 6 in. apart spread out from the points so that practically all the air between the points had a visible discharge in it.

In order to measure the number of ions per cc. in the air entering and leaving the plant chambers it was necessary to build a device which could be inserted in the air line and by which all of the ions could be discharged. As these were passing in at a constant rate, a constant current would result that could be measured on a galvanometer or electrometer. A Dolzalek

electrometer was used (capacity 93 e.s.u.) because, by inserting different capacities in parallel, it could be made flexible in use over the range of currents to be measured and because a galvanometer that was sufficiently sensitive for some of the small currents to be measured was not available.

The device through which the ionized air passed into the chamber is shown in figure 3. It consisted of a brass tube of the same size as the pipe entering the chamber. A brass rod was suspended longitudinally down the center of the tube from its middle by another rod passing up through two cakes of sulphur held by a glass tube with its lower end drawn in but not touching the brass rod, so that the surface of the sulphur would not become contaminated from dust, moisture, etc., in the air passing through the tube. The use of sulphur was found necessary. At first, glass alone was used, and there was a considerable leakage over 12 inches of glass tubing from which the rod was suspended at the top, due to surface moisture. The outside tube, which was completely insulated from connecting pipes, was charged up to the proper voltage by connection to one pole of a battery, the other of which was grounded.

A lead was taken off the suspended rod to one quadrant. Because of the strong electric waves given off by the spark discharges, which would greatly affect the electrometer, it was completely shielded in a galvanized iron box, the beam of light for reading passing through a screen. The lead from the suspended rod and the part projecting up above the brass tube was shielded by metal tubing. It was not found necessary to place screens across the ends of the brass tube because the suspended rod, placed only within 2 inches of each end, was found to be so completely shielded as not to affect the electrometer. Then, by first placing the quadrant with suspended rod and lead at ground potential and with the outer tube charged, all ions of similar charge were driven to the brass rod where they gradually charged the quadrant up to the potential of the outer tube. The rate at which the quadrant charged up, and thus the rate of turning of the needle as measured on the scale, depended on the number of ions available to carry the current across from the outside tubes to the rod, and also on the capacity of the quadrant, lead, and rod. Knowing the capacity of the system (found by the usual method of calibrating with a known capacity), the charge of a single ion, the number of centimeters of scale corresponding to a change of 1 volt in the potential of the quadrant, and the rate of flow of air in cubic feet per minute, the number of ions per cc. may be calculated as follows:

$$2 \times \frac{\dfrac{(Ce + Ce)\ (V/cm.) \times (\text{number of cm.})}{t}}{(4.77 \times 10^{-10}\ \text{e.s.u.})\ (30.48\ \text{cm.})^3 \times \dfrac{28.6\ \text{cu. ft. per min.}}{60}} = \text{no. of ions per cc.}$$

Explanation of symbols:

Ce = capacity of electrometer and leads (93 e.s.u.)

Cc = capacity of air condenser inserted in parallel

V/cm. = number of volts to which 1 cm. corresponds on scale placed at one meter = 1.2

Number of cm. = number of cm. taken for reading

t = time in seconds for the needle to move the above number of cm.

4.77×10^{-10} e.s.u. = one elementary unit of charge, *i.e.*, charge on one ion

$(30.48)^3$ = conversion factor for changing cubic feet to cubic centimeters

28.6 cu. ft. per min. = rate of flow of air per minute, divided by 60 to change to seconds

2 = factor for total number of ions as only one-half of the ions (as measurements showed there were equal numbers of + and − ions) are driven to the quadrant of the electrometer, the other half being neutralized by the charge of the tube.

It was found that charging the outside tube to 135 volts was sufficient to take out all of the ions at the rate of air flow used. Further increase in voltage gave no greater rate of movement of the needle. In fact, 90 volts gave, within a small percentage, the same reading as 135 volts.

In calibrating the electrometer several radon tubes were used as a constant source of ionization. These were suspended in the brass tube. It was found that the needle swung extremely rapidly and, in order to get the motion down so that readings could be made accurately, almost all of the tubes had to be removed. It was subsequently found that by placing the radon tubes at the entrance to the plant chamber (air flowing at the time) and the ionization chamber at the outlet, a higher reading was obtained than if the air had passed through sparks. In order to see how many ions per cc. could be produced with a flow of 28.6 cu. ft./min., a glass tube was coated on the inside with several hundred old radon tubes and another smaller glass tube suspended longitudinally in the larger tube, it being coated on the outside with radon tubes (fig. 4).

It was thus found that 1,100,000,000 ions per cc. could be produced at the near end with a flow of 28.6 cu. ft./min. That this measurement was correct was shown by placing three feet of pipe between the ionization chamber and the radon tubes. No current could be detected by the electrometer unless air was flowing through the pipe, showing that what was being measured was actually the number of ions per cc. and not the rate of production of ions by rays of some sort. At this point adding additional radon tubes did not increase the ionization perceptibly.

At the outlet of the chamber, owing to recombination of ions while passing through, the reading dropped so that only 21,000,000 ions per cc. were measured, but this is still a large number.

It was found that the Tesla coil discharge would give only about one-fifth of this number, and the maximum white spark discharge obtainable gave only about one-twelfth as many. This latter degree of ionization was obtained at a point distance of about 4.5 cm., when there was probably the greatest density of sparks and greatest potential drop per centimeter. Practically no ionization took place unless there was a spark or corona discharge. Sparking, and not the field alone, is evidently necessary to get a large degree of ionization by collision. This would indicate that in experiments with overhead wires little ionization takes place. The ionization readings remained constant and the maximum degree of ionization was used throughout the experiments using ionized air. Strangely enough, a spark discharge used as an auxiliary source with the radon tubes, decreased rather than increased the readings.

Owing to the fact that elaborate equipment would be needed to employ x-rays and eliminate errors and danger from their use, and that the tubes would not be constant sources, and could not be run for long periods, they were not tried.

Measurement and production of ozone

The air from the plant chamber was drawn through two Fisher wash bottles containing KOH solution and through two containing neutral KI solution at a constant known rate. This rate was determined by calibration of a capillary flow-meter and kept constant by regulation of the differential pressure. It was found, in calibrating, that if the differential pressure was set at a certain point, the time of flow of a certain volume of air could be predicted within a fraction of 1 per cent. The rate used was about one-half liter per minute. The iodine liberated according to the equation

$$2KI + O_3 + H_2O = 2KOH + I_2 + O_2$$

was titrated in acid solution according to the method described by Treadwell and Hall (**26**).

$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_2O_6$$

or 2 moles of $Na_2S_2O_3 = 1$ mole O_3. Thus knowing the volume of gas drawn through the absorption bottles and the number of cc. of 0.01 molar $Na_2S_2O_3$ solution used, the number of parts of O_3 is easily determined.

$$\frac{\frac{1}{2}\text{ number cc. of 0.01 M } Na_2S_2O_3 \times \frac{22{,}400}{1000 \times 100}}{\text{(Number of minutes of absorption) (cc. per min.)}} = \text{Parts of ozone per one part of air.}$$

(Corrected for standard conditions)

To produce O_3, and to be able to regulate the amount, a Siemens type of ozonizer was built (diameter 4 in.). Regulation of the concentration of ozone could be accomplished by changing the area of the outer layer of foil, by changing the potential, by changing the cross sectional open areas between the glass tubes or the amount of open area of the inside tube, thus causing more or less air to flow relatively between the tubes where it was ozonized by the strong alternating electric field. Twenty to thirty thousand volts could be used without sparking. The air, after being ozonized, was passed through the deionizer.

It was soon found that only small concentrations of O_3 were necessary and at none of the concentrations used was any N_2O_5 detectable by passing large volumes of the air through KOH and testing the resultant solution by several of the most sensitive qualitative tests for NO_3. All lower oxides that may be formed are immediately oxidized to N_2O_5 by O_3 (**4**).

Experimentation

Experiments 1 and 2

Thirty-three seedlings were transplanted into each chamber. The primary leaves were about one inch across. Lighting was for 16 hours daily. Ionization was at a maximum rate and was continuous, using the radon tube device. The sand was saturated and drained every twenty-four hours.

The plants grew rapidly and bloomed nineteen days after transplanting, and twenty-nine days after planting of the dry seeds in moist sand. Especially noteworthy was the uniform growth of plants and uniformity of time of blossoming in the two chambers. The leaves were unusually large and the internodes elongated, but there was practically no lodging. The plants were all about twenty-three inches high. The treated and control plants were identical in appearance. Also, there were no differences to be observed between the plants growing near the inlet and those near the outlet.

The chambers were alternated between the control and treated sets in the two experiments so that if there were any differences due to conditions in the chambers they would be discovered. There was, as shown, a large increase in dry weight, indicating that the plants grew considerably, and any effect due to the treatment should have been noticeable. As indicated in the last line of table I, the treated plants weighed 1.7 per cent. more in experiment 1, and 2.2 per cent. less in experiment 2 than the controls. There was thus no significant difference apparent from growing in highly ionized air.

Experiments 3 and 4

Since the previous experiments show no differences by using bright light, and as Henrici's results indicated that the effect on assimilation was more pronounced in dim light, it was decided to grow two sets as in experiments

TABLE I

RESULTS OF EXPOSING BEAN PLANTS TO IONIZED AIR
DATA FOR THIRTY-THREE PLANTS

	EXPERIMENT 1		EXPERIMENT 2	
	TREATED	CONTROL	TREATED	CONTROL
	gm.	*gm.*	*gm.*	*gm.*
Fresh weight, tops	406	393	375	387
Fresh weight, roots	88	95	77	82
Dry weight, tops	49.1	47.9	45	46.1
Dry weight, roots	6.8	7.1	5.2	5.3
Total dry weight	55.9	55.0	50.2	51.4
Weight of 33 seeds	10.94			
Weight of 33 seedlings, dry	7.3			
Percentage increase during period of treatment	766%	753%	688%	700%
Percentage difference of treated	+1.7%		−2.2%	

1 and 2, except that the bright lights (1000 watts) were on for only eight hours and were replaced with 100-watt bulbs for the remaining sixteen hours of each day. Under this changed condition, the plants did not grow as well. They were shorter, the leaves were smaller and seemed less succulent. A large percentage of the blossom buds aborted, the immature blossoms falling off before maturing. There were no apparent differences between the treated plants and controls. The dry weight of thirty-three plants was much less than before (table II). The figures in the table indicate how uniform the conditions of growth must have been.

EXPERIMENT 5

Again using ionized air two hours in the morning (9 to 11 A. M.) and two hours in the afternoon (3 to 5 P. M.), and lighting each case with two 1000-watt bulbs, as in experiments 1 and 2, no significant effect was obtained (table II).

TABLE II

RESULTS USING LIGHT OF LOW INTENSITY AND IONIZATION INTERMITTENTLY
DRY WEIGHTS FOR 33 PLANTS

EXPERIMENT	TREATED	CONTROL	PERCENTAGE DIFFERENCE
	gm.	*gm.*	%
3	34.0	33.4	+1.8
4	31.0	31.0	0.0
5	47.5	48.7	−2.5

In the above experiments with ionized air the radon tubes, rather than a spark discharge, were used because the high frequency could not be cut down to a point where the O_3 produced did not kill the plants. Using the blue-white sparks direct from the secondary, with condensers in parallel, it was found that also here, unless the sparking and potential were cut down to a point where relatively very few ions were produced, the ozone was still excessive. Radon produced no detectable O_3.

Experiment with ozone

In this experiment the procedure was to start with applications of relatively high concentrations of ozone, 1 part in 50,000 to 100,000 of air, in which case results were visible in a few minutes, and then decrease the concentration until either no toxic or no beneficial effects were indicated. Bean plants were used as before.

It was found, first, that concentrations of one part of ozone in 100,000 of air resulted in a wilting of the leaves in 15 to 30 minutes. The lower and older leaves succumbed first, while the very youngest were not affected. The ozonizer was then disconnected and the plants allowed to recover, if still capable of doing so. The wilted leaves did not recover but became bleached, giving a yellow enameled appearance. In those less affected there remained green stripes along the larger veins, while in others the part of the leaf nearest the stalk appeared unaffected.

The topmost younger and not fully expanded leaves were unaffected. The plants were allowed to continue to grow, but, although they bloomed at the same time as the controls, they were stunted and their dry weight, including the bleached leaves, was only three-fifths as much.

With concentrations of one part of ozone in 1,000,000 of air applied only for periods of 30 minutes twice daily, the plants presented a somewhat stunted and dried up appearance, and in eight days brown spots appeared on the older leaves. If this concentration was applied continuously, the plants showed a slightly withered appearance of the leaves and brown spots appeared on the second day.

With a concentration of 1 part in 3,000,000 of air there was no noticeable desiccated appearance of the leaves, and the spotting occurred in four days, always on the older leaves. If the application was continued after this, the spots increased in size and the leaves dried up and fell off, the younger leaves gradually being affected, and then the stem.

With concentrations of 1 part in 6,000,000 of air the spotting did not occur until the twelfth day, the plants appearing normal until then.

With a concentration of 1 part of ozone in 8,000,000 of air, a slight speckling was shown in three weeks.

Another set of plants was grown at a concentration of 1 part of ozone in 10,000,000 of air, applied during two one and one-half-hour periods daily,

and another set at a concentration of one part in 17,000,000 of air continuously for eighteen days. In neither case was there any noticeable change in appearance nor significant difference in the dry weights which were 41.8 and 43.4 gm. for the two treated lots respectively, and 42.9 gm. for the control.

In the above cases of spotting, closer examination showed that the spots originated mostly in the islets between the smaller veins, where there was also a greater density of stomata. The affected cells appeared darkened, desiccated, and collapsed. Similar effects at like concentrations were found for such plants as *Abutilon* and *Coleus*.

Since it was noted that ozone in sufficient concentration had a bleaching action on chlorophyll and it seemed reasonable to assume that such a strong oxidizing agent might accelerate respiratory or aging processes, it was decided to try the effect of different concentrations of ozone on the rate of ripening of green bananas and tomatoes. Results indicated that no change occurred in the rate of ripening of bananas or tomatoes. The conditions were such that the fruit ripened excellently, but unless sufficient concentrations of ozone were used to produce brown spots, no effect was noted. Much higher concentrations of ozone (1 part in 30,000) were required to spot the fruits than was found to so affect the leaves, probably due to the lack of stomata and greater cutinization of the epidermis of the fruits.

The Tesla coil and discharge box being available, it was thought advisable to determine what effect, if any, the Tesla discharge might have on dry and germinated seeds. Consequently dry seeds and germinated seedlings of corn and sunflower were treated for periods of various lengths. The seeds and seedlings were suspended on a heavy sheet of paper between the discharge points of the two plates. Only the fine spray sparks were used and not the cracking ones, also various point distances were used. They were thus subjected to an intense high frequency field and a high concentration of ozone. No effect was noted on the growth of the plants unless the seeds were exposed for as long as 12 minutes at a point distance of 4 cm. and frequency of 4,500,000 cycles per second. This treatment resulted in stunted plants, most of which did not continue to grow after eight to ten days, although none of the seeds were killed outright by the treatment.

Discussion

The writer thus finds that the results of KOERNICKE, and of LIPPERHEIDE to the effect that ionization of the air promotes growth are not confirmed. The writer used a much higher degree of ionization, both continuously and intermittently, than did LIPPERHEIDE; also, the degree of ionization varied greatly between the entrance and exit, and no effect of ionization gradient was noted. It is not likely that such low degrees of ionization as LIPPER-

HEIDE must have used could have had any effect on growth. His lack of descriptive information makes it impossible to duplicate his conditions. We cannot use increased ionization of the air, therefore, to explain some of the previous results of electroculture but must fall back on criticism of control of conditions.

In regard to ozone, the necrosis of only local areas at first, and these on the older leaves, is difficult to explain. Usually with toxic gases the younger parts are first attacked. Microscopic examination showed that the spotting occurs in areas where the stomata are more numerous. It is suggested that the action of ozone is partly the saturation of lipoid compounds in the cell membranes, resulting in loss of semipermeability, wilting, oxidation of cell contents, and death of the cell.

Since it is difficult to summarize completely the errors in previous work that indicate opposite results to those described in this paper without too much repetition, the reader is referred to the section on results of previous investigators. It may be said here, however, that major mistakes resulted from not isolating the factors, not measuring the conditions, measuring rates of production of ions rather than concentration (actual number per cc.), not controlling other conditions, such as humidity, light, etc., not using a sufficient number of plants, and the use of very weak sources of ionization. The reader is requested to check the methods developed in this paper to assure himself that the technic here used did not admit of any conditions that would invalidate the results.

It may be said that in the experiments herein described plants for the first time have been grown for a period in conditions where the factors of ionization of the air, and ozone, were isolated. The writer contends that examination of growth over a period (from seedling to blossoming) is more likely to show the effects of these factors than measurements of assimilation or respiration over short, intermittent periods.

Summary

1. Plants grown in highly ionized air (1,100,000,000 ions to 21,000,000 per cc.) showed no significant changes in appearance or dry weight over controls, whether the treatment was applied continuously or intermittently with bright or with dim illumination.

2. Ozone is toxic and produces necrotic spots with final desiccation of the leaf in concentration of 1 part in 1,000,000 to 1 in 8,000,000 of air.

3. Lower concentrations of ozone, which are still easily detectable by odor, produce no stimulation of growth or other effects on the appearance of the plants.

4. Higher concentrations, about 1 part in 100,000, produce prompt wilting and bleaching, the older leaves succumbing long before the younger upper ones.

5. Ripening processes in tomato and banana are not accelerated by O_3. High concentrations produce spotting. These are much higher than concentrations required for spotting of leaves.

6. A Tesla discharge had no effect on dry or germinated seeds unless exposure was for twelve minutes, when stunted plants that did not develop were produced.

7. A method is developed that would be applicable to a study of the effects of different gases on plants under controlled conditions.

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INTENSITY OF REMOVAL OF CATIONS FROM COTTON, CORN, AND SOY BEAN TISSUE BY FRACTIONAL ELECTRODIALYSIS[1]

H. P. Cooper, W. R. Paden, and R. L. Smith

(WITH ONE FIGURE)

Introduction

In a consideration of the characteristics of various plants a number of empirical studies have been made on the soil fertility requirements, shade tolerance, and food value of plants grown under varying environmental conditions (**2, 3, 4, 5**). There seemed to be some correlation between the relative strength of ions required by plants for optimum growth and the quality of light required. Certain plants which grow successfully in the shade may utilize relatively more of the weak nutrient ions. It might be expected that plants which have the ability to utilize weak nutrient ions and tolerate shade would synthesize organic compounds of relatively low quality energy value. These general observations lead to a consideration of the application of certain of the modern concepts, which have been useful in chemistry and physics, to soil fertility and mineral nutrition studies of plants.

Since every form of energy may be considered as compounded of two factors, one the intensity factor and the other the capacity factor, it is desirable to know the relative importance of these two factors in biological reactions. The data reported are concerned with the intensity factor and only incidentally with the capacity or quantity factor. It is believed that the life processes are more intimately concerned with the intensity factor of energy.

The recent interest in the studies of soil colloids has led to new concepts concerning soil chemistry and the availability of nutrients in the soil. In a recent paper (**4**) special consideration has been given to the intensity of removal of added cations from soil colloids by electrodialysis. It was found that there is a differential in the intensity of removal of certain cations from soils, and that there is a correlation between the intensity of removal and the strength of ions. The strongest ions were removed most rapidly from the soil. The oxidation-reduction potential values were found to be useful in predicting the intensity of removal of cations from soils by electrodialysis (**3, 4, 5**). Since there is apparently a correlation between the intensity of removal of some nutrient ions from the soil and the intensity of their absorption by certain plants, one might expect that there would also

[1] Contribution from the Department of Agronomy, South Carolina Agricultural Experiment Station, Clemson, South Carolina. Technical contribution no. 53 (new series).

be a differential in the intensity of removal of cations from plant tissue by electrodialysis. In order to secure data on this subject electrodialysis studies were made on the tissue of a number of crop plants.

Electrodialysis studies of plant tissue

The intensity of absorption of nutrient ions by plants would be expected to be correlated with the intensity of their removal from soil by electrodialysis. It is well known that certain plants differ markedly in the ions which they selectively absorb. As there is such a variety of conditions determining the intensity of absorption of nutrient ions, such as permeability of root membrane, utilization of ions, and formation of insoluble compounds, it is very difficult to determine the order of intensity of absorption of ions by different plants. However, it is believed that fractional electrodialysis studies of plant tissue will serve to establish the general relationship between the relative strength of ions and their intensity of absorption, mobility in tissues, and utilization by certain plants.

In order to determine the intensity of removal of cations, fresh samples of cotton, corn, and soy bean tissue were electrodialyzed in a Löddesöl electrodialysis apparatus (**8**), and analyses of the diffusate collected at successive intervals for a total of 29 to 32 hours. The usual analytical methods employed in soil studies were used for the determination of cations removed from the plant tissue (**12**). The uranyl-zinc-acetate method (**1**) was used in some of the determinations of the sodium content of the diffusate.

The data secured in these studies are shown in table I and in figure 1. The milliequivalents (ml. eq.) removed during each extraction period per 100 gm. of fresh plant material and the percentage of the total milliequivalents for each element are included. The percentage removal of the various metals for each extraction period shows that there is a differential in the intensity of removal of cations from plant tissue.

The percentage of the total amounts of potassium, sodium, calcium, and magnesium extracted from cotton tissue during the first hour period was 91.40, 49.57, 31.10, and 1.60 per cent., respectively. During the 3- to 5-hour extraction period, the above quantitative relations were reversed. The percentage of the total amounts removed was 0.50, 5.18, 11.90, and 60.20 per cent., respectively.

In the studies on corn and soy bean tissue, the first four extraction periods were one-quarter of an hour each. The fifth and sixth periods were one-half an hour each. The percentage of the total amounts of potassium, sodium, calcium, and magnesium extracted from corn tissue during the first quarter-of-an-hour period was 81.55, 22.23, 13.19, and 2.25 per cent., respectively. Likewise the figures for the studies on soy bean tissue were 43.09, 36.47, 1.73, and 1.46 per cent., respectively.

TABLE I

INTENSITY OF EXTRACTION OF CATIONS FROM FRESH COTTON, CORN, AND SOY BEAN TISSUE BY FRACTIONAL ELECTRODIALYSIS IS CORRELATED WITH THE ELECTROMOTIVE SERIES

Period of extraction	Milliequivalents per 100 gm. of fresh tissue							
	K		Na		Ca		Mg	
hr.	*ml. eq.*	%	*ml. eq.*	%	*ml. eq.*	%	*ml. eq.*	%
	Cotton							
0– 1	7.397	91.40	1.750	49.57	18.360	31.10	0.200	1.60
1– 2	0.459	5.70	0.065	1.84	18.280	31.00	0.280	2.20
2– 3	0.028	0.40	0.116	3.28	8.440	14.40	0.480	3.80
3– 5	0.038	0.50	0.183	5.18	7.000	11.90	7.580	60.20
5– 7	0.002	0.00	0.297	8.41	2.080	3.50	3.180	25.20
7– 9	0.015	0.20	0.189	5.35	1.410	2.30	0.320	2.50
9–11	0.023	0.30	0.207	5.86	1.160	2.00	0.160	1.30
11–13	0.043	0.50	0.146	4.13	0.840	1.40	0.160	1.30
13–21	0.056	0.60	0.342	9.68	0.720	1.20	0.160	1.30
21–29	0.033	0.40	0.235	6.65	0.800	1.20	0.080	6.30
Total extracted	8.094	100.00	3.530	100.00	59.090	100.00	12.600	100.00
	Corn							
0.00–0.25	2.228	81.55	0.081	22.23	0.592	13.19	0.079	2.25
0.25–0.50	0.451	16.51	0.125	34.72	1.923	42.84	0.039	1.11
0.50–0.75	0.053	1.94	0.053	14.75	1.005	22.39	0.720	20.50
0.75–1.00	.....	.....	0.072	19.98	0.326	7.26	1.174	33.44
1.00–1.50	.....	.....	0.030	8.32	0.209	4.66	0.987	28.10
1.50–2.00	.....	.....	.....	.....	0.107	2.38	0.414	11.79
2– 3	.....	.....	.....	.....	0.128	2.85	0.030	0.85
3– 4	.....	.....	.....	.....	0.122	2.72	0.030	0.85
4– 6	.....	.....	.....	.....	0.056	1.25	0.039	1.11
6– 8	.....	.....	.....	.....	0.021	0.46	.....	.....
8–32	Trace	.....	Trace	.....	Trace	.....	Trace	.....
Total extracted	2.732	100.00	0.361	100.00	4.489	100.00	3.512	100.00
	Soy bean							
0.00–0.25	2.059	43.09	0.217	36.47	0.173	1.73	0.049	1.46
0.25–0.50	2.236	46.80	0.274	46.05	0.887	8.85	0.020	0.60
0.50–0.75	0.435	9.10	0.074	12.44	2.387	23.81	0.030	0.90
0.75–1.00	0.049	0.01	0.030	5.04	1.387	13.83	0.049	1.46
1.00–1.50	Trace	.....	Trace	.....	1.780	17.75	0.030	0.90
1.50–2.00	.....	.....	.....	.....	1.224	12.21	0.928	27.75
2– 3	.....	.....	.....	.....	0.796	7.94	1.236	36.93
3– 4	.....	.....	.....	.....	0.424	4.22	0.543	16.22
4– 6	.....	.....	.....	.....	0.587	5.85	0.087	2.60
6– 8	.....	.....	.....	.....	0.219	2.18	0.138	4.12
8–10	.....	.....	.....	.....	0.051	0.51	0.069	2.06
10–12	.....	.....	.....	.....	0.036	0.36	0.030	0.90
12–14	.....	.....	.....	.....	0.021	0.21	0.039	1.17
14–16	.....	.....	.....	.....	0.010	0.10	0.030	0.90
16–24	.....	.....	.....	.....	0.030	0.30	0.039	1.13
24–32	.....	.....	.....	.....	0.015	0.15	0.030	0.90
Total extracted	4.779	100.00	0.595	100.00	10.027	100.00	3.347	100.00

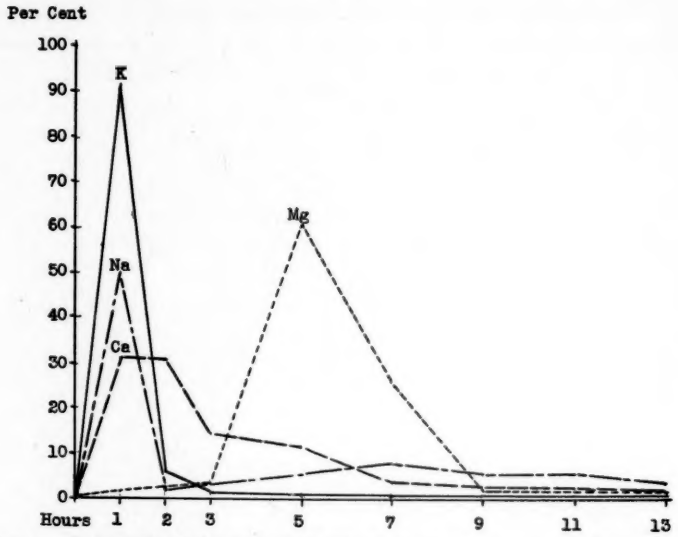

FIG. 1. Percentage of the total milliequivalents of cations removed from 100 gm. of fresh cotton tissue at various intervals by fractional dialysis.

The order of intensity found in these studies is in agreement with the findings of MOORE, REEVES, and HIXON (9), who reported that most of the potassium and sodium was removed from apple tissue during the first hour of electrodialysis. NELLER (10) found that potassium was readily removed from apple tissue.

The data from the fractional electrodialysis studies of plant tissue show the same order of intensity of removal of cations as was found in the soil studies (4). The establishment of the correlation between the oxidation-reduction potential values, the ionization-potential values, and the intensity of removal of cations from soil and plant tissue may be very significant in certain soil and plant nutrition studies. As there is a close relation between the intensity of removal of ions from the soil by electrodialysis and the intensity of their absorption by certain plants, and also the quality of radiant energy required for the assimilation of certain nutrient salts, some oxidation-reduction reactions occurring in growth processes may be of special interest.

Some common oxidation-reduction reactions occurring in physiological processes

Since there is apparently a close relation between the intensity of removal of ions from soil colloids and plant tissue by electrodialysis and the relative

strength of ions, it is interesting to consider the significance of these relations in plant nutrition studies. As there is apparently a close correlation between the strength of ions and the intensity of their absorption by plants, it would seem reasonable to expect that there would be a close correlation between the relative strength of ions required by plants for optimum growth, the quality of light required for their assimilation, and the food value or quality of energy supplied by certain organic compounds.

Some of the important energy relationships represented by the light absorption bands of chlorophyll are in close agreement with the free-energy decrease in the formation of certain common nutrient salts, and with the energy required for the reduction of common nutrient anions such as nitrates, borates, carbonates, and phosphates (3).

Most plant life is dependent upon the nutrient salts and the absorption of light energy from the sun. It might be expected that there would be a

TABLE II

SOME COMMON EQUIVALENT OXIDATION-REDUCTION REACTIONS OCCURRING IN CERTAIN PHYSIOLOGICAL PROCESSES WHICH TAKE PLACE ON APPROXIMATELY THE SAME ENERGY LEVEL* APPROXIMATE EQUIVALENT ENERGY IN SOLAR SPECTRUM RANGES FROM 0.41 TO 4.20 VOLT-FARADAYS, WHICH IS EQUIVALENT TO 30,000 TO 2900 ÅNGSTROMS

REACTION AND EQUIVALENT ENERGY INVOLVED = VOLT-FARADAYS							EQUIVALENT LIGHT ENERGY	
							ÅNGSTROMS	COLOR
NO_3^-	=	NO_2^-	+	O	+	1.05	11757	Infrared
$H_2O + O$	=	H_2O_2			+	1.03	11985	Infrared
$CuCl_2$	=	Cu	+	2Cl	+	1.01	12223	infrared
Mn^{++}	=	Mn^{+++}			+	1.07	11537	Infrared
BO_3^{---}	=	BO_2^-	+	O	+	1.77	6974	Red
$FeCl_2$	=	Fe	+	2Cl	+	1.78	6935	Red
$H_2AsO_4^-$	=	$H_2AsO_3^-$	+	O	+	1.98	6235	Orange
$CrCl_2$	=	Cr	+	2Cl	+	1.90	6497	Orange
$H_2CO_3^-$	=	$H_2CO_2^-$	+	O	+	2.85	4332	Violet
$MgCl_2$	=	Mg	+	2Cl	+	2.85	4332	Violet
$H_2PO_4^-$	=	$H_2PO_3^-$	+	O	+	3.35	3685	Ultraviolet
CaC_2O_4	=	Ca	+	C_2O_4	+	3.35	3685	Ultraviolet
$H_2PO_3^-$	=	$H_2PO_2^-$	+	O	+	3.79	3257	Ultraviolet
$CaCl_2$	=	Ca	+	2Cl	+	3.85	3206	Ultraviolet
$CaHPO_4$ decomposition potential						4.20	2939	Ultraviolet
Effective rays in preventing rickets						4.16	2968	Ultraviolet

* These values were obtained from International Critical Tables 5: 169–207. 1929.

close relation between the salts used by plants and the quality of radiant energy absorbed by them.

Little attention has been given to the definite correlation between the quality of the free-energy decrease in the formation of nutrient materials and light absorption by plants. Certain data have been compiled to suggest to those interested in photosynthesis studies the possible significance of these relationships in a study of the soil fertility and light requirements of plants and the quality of the energy supplied by certain food materials.

Some of the common oxidation-reduction reactions occurring in nutritional processes are compiled and included in table II. Since the higher plants depend upon energy from the sunlight for growth, it is desirable to consider the energy relationships involved in terms of volt-Faradays, wave length in Ångstroms, and color of light. All of these values can be converted from one to another very easily by the use of Einstein's photochemical equation. This equation and the factors used in conversion of one mode of expression of energy into another were taken from International Critical Tables (**7**) and RICHTMYER (**11**), and are as follows:

$$E = Nh\nu = Nh\frac{c}{\lambda}.$$

$$\text{Ångstroms} = \frac{12345}{\text{volt-Faradays}}$$

$$\text{Volt-Faradays} = \frac{\text{gram calories}}{23058}$$

$$\text{Gram calories} = \text{volt-Faradays} \times 23058.$$

The conversion of the energy values involved in various reactions into equivalent volt-Faradays, Ångstroms, and color of light shows more clearly the relationship between each of these values.

The reactions which occur on approximately the same energy level, and which may be effective in bringing about certain oxidation-reduction reactions necessary in physiological processes, are arranged in groups. It has been observed that on certain soils relatively high in nitrates there has been a marked response to application of copper, and in some instances to manganese. These responses are very probably related to their effect upon oxidation-reduction reactions. The greatest growth response observed from additions of boron to nutrient media seems to be in complexes where iron may not be readily available. There is also a relation between magnesium chloride and the reduction of the carbonate ion, which is a very important reaction in photosynthesis. Some of the important reactions, such as the reduction of nitrates to nitrites, the formation of hydrogen peroxide, the free energy decrease in the formation of cupric chloride ($CuCl_2$), and the change

of valence of manganese from Mn^{++} to Mn^{+++} are on approximately the same energy level. Likewise there is a close relation between the reduction of the borate ion to the borite ion and the free-energy decrease in the formation of ferrous chloride ($FeCl_2$); between the reduction of the carbonate ion to the carbonite ion and the free-energy decrease in the formation of magnesium chloride ($MgCl_2$); and between the reduction of the phosphate ion to the phosphite ion and the free-energy decrease in the formation of calcium oxalate (CaC_2O_4). The decomposition potential of di-calcium hydrogen phosphate ($CaHPO_4$) and the rays effective in the prevention of rickets in animals under certain conditions require approximately the same quality of energy.

It is believed that there is a definite relation between these reactions which take place at approximately the same energy level and that a careful study of these reactions will be of great value in solving some of the complex problems in nutrition. One might consider the close agreement between these values as a simple coincidence and that they need not necessarily bear any significant relationship one to the other. However, the possibility that these reactions may have a definite relationship would seem to merit careful consideration.

As there is a direct correlation between the strength of atomic metallic ions and their removal from soils by electrodialysis as well as their selective absorption by plants, a correlation would be expected between the optimum strength of nutrient ions required by plants, the quality of light required for their normal growth, and their shade tolerance. It is interesting to note that there is a very close correlation between the energy relationships represented by the light absorption bands of chlorophyll, the decomposition or discharge potentials of certain nutrient salts, and the quality of the energy necessary for the reduction of some common nutrient anions such as nitrates, borates, carbonates, and phosphates.

The relationships discussed in this paper are what one might expect from the known physical and chemical properties of the materials considered. A utilization of the modern concept in physics and chemistry to biological problems will make it possible to do more inductive research instead of depending largely upon results secured from empirical methods.

Summary

1. Samples of plant tissue were electrodialyzed in order to determine the intensity of removal of the various metals. The data presented illustrate very clearly the differential in the intensity of removal of various metals from plant tissue. The strongest ions are removed most readily from the plant tissue. Large proportions of the strong potassium and sodium ions were found in the first fractions of the diffusate. The fractional electrodi-

alysis studies on the intensity of removal of metallic ions from soils and from plant tissue, as would be expected, are in complete agreement.

2. A definite relationship exists between the intensity of removal of nutrients from the soil and the intensity of their absorption by plants. Therefore, it would seem logical to expect a definite relationship between the strength of ions used by plants, the quality of light required for their optimum growth, and the food value or quality of energy supplied by certain organic compounds.

3. Compiled data suggest that there is a relationship between certain common oxidation-reduction reactions and the free-energy decrease in the formation of certain nutrient salts, and also between the energy required for the reduction of certain nutrient anions, such as nitrates, borates, carbonates, and phosphates, and the quality of the light necessary for the optimum assimilation of the various nutrient materials by plants.

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LEAF TEMPERATURES OF SUGAR CANE

C. F. MORELAND

Since the results in the literature are at considerable variance regarding leaf temperatures with reference to the surrounding air temperature, the writer thought it would be of interest to check some leaf temperatures in a different section of the country from those previously reported. The investigations of CURTIS (**2**) were performed at Berkeley and Riverside, California, and those of CLUM (**1**) at Ithaca, New York. The results of MILLER and SAUNDERS (**5**) were obtained in Kansas, and those of EATON and BELDEN (**4**) in Arizona.

Material and methods

The writer's experiments were carried out in the fall of the last year at Baton Rouge, Louisiana. Some of the experiments were performed under field conditions while others were with cut plants in the laboratory.

Sugar cane, variety P.O.J. 213, was used as experimental material. The plants used were in the variety test plots at the Louisiana Experiment Station, Baton Rouge. At the time of the experimentation the plants were 8 to 10 feet high.

Thermocouples made of copper and constantan wires (30 gauge) were used for making the temperature readings. The temperatures were ascertained in two different ways. Under field conditions, the standard junctions of two thermocouples were fastened against the bulb of a thermometer by means of a small rubber band. The thermometer together with the thermocouples was then placed in a thermos bottle filled with water having a temperature about that of the air. The other junction of each thermocouple was placed as follows: No. 1 was passed through the leaf and the wires bent in such a way that the junction was in contact with the lower epidermis of the leaf, or, in some cases, no. 1 was inserted into the mid-vein from the lower side of the leaf. No. 2 was allowed to hang in the shade of the box used to convey the apparatus, or, in some cases, was allowed to hang in the direct sunlight. The two leads from the thermocouples were attached to a multiple switch and thence to a galvanometer (Leeds & Northrup no. 2420 B). With this outfit, the direction and amount of deflection of the galvanometer were tabulated. The temperature of the water in the thermos bottle, being near that of the air, remained quite constant for the duration of each series of readings (about one hour).

After the readings had been obtained (in the field) the galvanometer deflections were then calibrated (in the laboratory) against each thermocouple. The standard junctions were placed in water adjusted to the same

temperature as under the experimental conditions. The other junctions were also fastened to the bulb of a thermometer as the standard junctions. These were placed in a beaker of water, the temperature of which was adjusted at half-degree intervals until the deflections of the galvanometer covered the entire range of the scale. From these deflections a graph was charted for each thermocouple and the deflections under field conditions were translated into temperatures. The accuracy of this method depends largely on the accuracy of the thermometer used, but, as CURTIS (**2**) has pointed out, the changes in leaf temperatures are so rapid and great that small differences in temperature (1/10° to 1/20° C.) are small enough to be of little importance.

With this outfit deviations in temperature of only about 5° C. (2.5° above and 2 to 5° C. below the standard junction) could be determined. Since differences in temperature were often greater than this, the method was modified and a Leeds & Northrup student type potentiometer was used in conjunction with a standard cell and with the galvanometer described above.

Since this apparatus was not mounted in such a way as to be portable, the top halves of cane plants were cut, placed immediately in water, and then brought to the laboratory. The cut plants were placed directly in front of a large south window which was open from top to bottom. There was no artificial heat being used in the building at the time. Owing to the angle of the sun's rays at the time of the year the experiments were conducted, the plants were well exposed to direct sunlight in the middle of the day.

In using the potentiometer the standard junctions of the thermocouples were placed in a thermos bottle filled with crushed ice and distilled water.

Two sets of temperature readings were obtained under field conditions and three sets under laboratory conditions.

Experimental results

In the afternoon of October 16, 1936, the weather was clear but very windy. October 23 was again clear but not nearly so windy as the previous week. The results of the temperature readings taken in the field on these two dates are given in tables I and II. It is to be noted that leaf temperatures are more variable under windy conditions than when the air is relatively still; also, that there is a greater difference between the leaf temperature and the surrounding air when the air is still. On October 16, when the wind was blustery, the temperature changes were so rapid that it would have been necessary to take simultaneous readings of leaf and air temperatures in order to make them entirely comparable for a given instance, as the temperature changes were very rapid. The galvanometer deflection would change 10 to 15 mm. (1 to 1.5° C.) in one or two seconds.

TABLE I

COMPARISON OF TEMPERATURE OF SUGAR CANE LEAVES AND SURROUNDING AIR UNDER FIELD CONDITIONS ON A CLEAR WINDY (VERY) DAY. RELATIVE HUMIDITY 59 PER CENT. READINGS 3:00 TO 4:00 P.M., OCTOBER 16, 1936. AVERAGE BASED ON 25 READINGS. LEAF TEMPERATURE OBTAINED BY THREADING THERMOCOUPLE INTO MIDRIB FROM LOWER SIDE OF LEAF

THERMOCOUPLE NO.	RELATION TO SUNLIGHT		TEMPERATURE		
	DIRECT SUNLIGHT	SHADE	MINIMUM	MAXIMUM	AVERAGE
			°C.	°C.	°C.
1	leaf	.....	31.00	32.50	32.10
2	air	.....	29.50	31.50	31.15
1	.....	leaf	27.20	27.50	27.30
2	.....	air	28.00	28.75	28.30

TABLE II

COMPARISON OF LEAF TEMPERATURES AND SURROUNDING AIR UNDER FIELD CONDITIONS ON A CLEAR AND SLIGHTLY WINDY DAY. RELATIVE HUMIDITY 63 PER CENT. READINGS 3:00 TO 4:00 P.M., OCTOBER 23, 1936. AVERAGE BASED ON 10 READINGS. THERMOCOUPLE THREADED THROUGH LEAF IN CONTACT WITH LOWER EPIDERMIS

THERMOCOUPLE NO.	RELATION TO SUNLIGHT		TEMPERATURE		
	DIRECT SUNLIGHT	SHADE	MINIMUM	MAXIMUM	AVERAGE
			°C.	°C.	°C.
1	leaf	.....	32.8	33.5	33.3
2	air	.....	27.1	27.5	27.4
2	.....	air	25.1	25.4	25.2

In the laboratory the temperature readings were taken under three sets of conditions. The first set of readings was taken on a clear day between 3:00 and 4:00 P.M. on November 3 (table III). Due to the season of the year and the time of the day, the sun's rays were coming through the open window very obliquely. The next two sets of readings were taken between 11:00 and 12:00 A.M., when the sun was almost directly in front of the window. However, the weather conditions were quite different on these last two days. On November 18, the day was partly cloudy, and sunshine alternated frequently with cloudy conditions owing to small patches of clouds passing in front of the sun. Also at times the clouds were so thin that the sun would partly shine through them (a condition described as hazy in table IV). November 20 was a clear, quiet day. The temperature readings are given in table V.

TABLE III

COMPARISON OF TEMPERATURES OF SUGAR CANE LEAVES AND SURROUNDING AIR IN THE LABORATORY, IN FRONT OF AN OPEN WINDOW, ON A CLEAR DAY. RELATIVE HUMIDITY 86 PER CENT. READINGS 3:00 TO 4:00 P.M., NOVEMBER 3, 1936. AVERAGE BASED ON 12 READINGS. THERMOCOUPLE THREADED THROUGH LEAF IN CONTACT WITH LOWER EPIDERMIS

THERMOCOUPLE NO.	RELATION TO SUNLIGHT		TEMPERATURE		
	DIRECT SUNLIGHT	SHADE	MINIMUM	MAXIMUM	AVERAGE
			°C.	°C.	°C.
1	leaf	.....	25.15	26.25	25.49
2	air	.....	23.75	25.25	24.95
1	.....	leaf	23.25	23.50	23.41
2	.....	air	24.00	25.00	24.46

TABLE IV

COMPARISON OF TEMPERATURES OF LEAVES OF CUT SUGAR CANE PLANTS AND SURROUNDING AIR IN THE LABORATORY ON A PARTLY CLOUDY DAY. RELATIVE HUMIDITY 63 PER CENT. READINGS 11:00 A.M. TO 12:00 M., NOVEMBER 18, 1936. THERMOCOUPLE THREADED THROUGH LEAF IN CONTACT WITH LOWER EPIDERMIS

THERMOCOUPLE NO.	RELATION TO SUNLIGHT			NO. OF READINGS	TEMPERATURE		
	DIRECT SUNLIGHT	SHADED BY CLOUD	HAZY		MINIMUM	MAXIMUM	AVERAGE
					°C.	°C.	°C.
1	leaf	.....	.....	3	28.25	29.15	28.60
2	air	.....	.....	3	24.60	26.25	25.70
2	.....	air	.....	8	22.90	24.90	23.56
1	.....	leaf	.....	8	22.50	23.50	23.24
1	.....	.....	leaf	7	23.50	26.90	24.46
2	.....	.....	air	7	23.40	26.00	24.25

Discussion

The temperatures found for the sugar cane leaves, as compared with the surrounding air, were more in agreement with the temperature readings obtained by CURTIS and CLUM than with those of MILLER and SAUNDERS and of EATON and BELDEN. The writer did not find leaf temperatures in direct sunlight, however, to be as much in excess of the surrounding air as did CLUM (**1**) or CURTIS (**2**), who often found leaf temperatures 10° above the air temperatures. As shown in table V, the greatest average difference observed by the writer was 8.5° C. This difference was even less on a very windy day (4.5° C.). CURTIS (**2**) has suggested some probable reasons why

TABLE V

COMPARISON OF TEMPERATURES OF SUGAR CANE LEAVES AND SURROUNDING AIR IN THE LABORATORY IN FRONT OF AN OPEN WINDOW ON A CLEAR DAY. RELATIVE HUMIDITY 51 PER CENT. READINGS 11: 00 A.M. TO 12: 00 M., NOVEMBER 20, 1936. LEAF TEMPERATURE OBTAINED BY THREADING THERMOCOUPLE THROUGH LEAF AND IN CONTACT WITH LOWER EPIDERMIS, OR THREADED INTO MIDRIB FROM LOWER SIDE OF LEAF

THERMOCOUPLE NO.	RELATION TO SUNLIGHT		NO. OF READINGS	TEMPERATURE		
	DIRECT SUNLIGHT	SHADE		MINIMUM	MAXIMUM	AVERAGE
				°C.	°C.	°C.
1	leaf (on surface)		15	31.25	38.25	34.91
1	leaf (in midrib)		10	30.00	36.75	33.63
2	air		15	27.75	31.25	29.54
2		air	10	25.75	27.00	26.41
1		leaf (on surface)	10	25.00	26.25	25.61

the data of some workers are at variance with those of others. He points out that the data of different investigators were obtained under different conditions of humidity and that this may influence leaf temperatures through its effect on infrared radiation. He points out also that different methods of obtaining the air temperature were used, and that great changes in the temperature of leaves in direct sunlight may be brought about by changes in rates of air flow. It seems to the writer that these differences in air movements, especially under field conditions, are significant. On October 16, when the wind was brisk, the average difference between the leaf temperature and the air (shade) was about 3° C., with a maximum difference of only 4.5° C. On November 20, when the air was still, the average difference between the leaf temperature and the air (shade) was 8.5° C. or 7.22° C. in the midrib, with a maximum difference of about 12° C. or 11° C. in the midrib. As stated previously, these differences were probably not as great as this for any given instant of time, for with any two contiguous readings of the leaf and air temperatures the greatest difference observed was 6.25° C. There was not a great deal of difference in relative humidity on these two days.

Of course, in comparing these two sets of data it must be borne in mind that the first were obtained under field conditions while the latter were obtained in the laboratory where cut plants were used. While precautions were taken to disturb the transpiration rate of the cut plants as little as possible, there is a possibility that the rate may have been reduced owing to closing of the stomata. The plants showed no signs of wilting for the duration of the experiment and were noted to be in good condition two days after

the experiment. The differences could hardly have been due to difference in relative humidity because the higher temperatures, as compared with the surrounding air, were obtained when the relative humidity was lower. The results obtained under two different conditions in the field on October 16 and 23, also indicate that air movements have more to do with leaf temperature, as compared with the surrounding air, than differences in relative humidity. It is true that the greater differences between leaf and air temperatures were observed with a higher relative humidity; but the difference in the relative humidity of these two dates was only 4 per cent. The average temperature difference between the leaf and air on October 16 (very windy day) was about 3° C. while on October 20 (slightly windy) the average difference was 8.1° C. As has been pointed out by CURTIS (3), the transpiration rate should have been greater under the condition of the higher humidity because the 5°-C. rise within the leaf in excess of that within the leaves under the lower relative humidity should more than offset the 4 per cent. difference in relative humidity. As shown in tables I and II the positions of the thermocouples for obtaining leaf temperature were different. In the first case the thermocouple was inserted into the midrib while in the second case it was in contact with the lower epidermis. This difference in position of the thermocouple may account for about 1 to 1.5° C. of the excess of the second readings over the first as compared to the surrounding air. This is shown in table V.

This point is further emphasized by the fact that the difference in temperature between the leaf in the shade and the surrounding air was not very different with the difference in relative humidity.

Summary

1. Environmental conditions such as air movements seem to alter considerably the sugar cane leaf temperature as compared with the surrounding air. With strong movements of air about the leaf, the difference is slight (1° to 2° C.), but in still air the leaf in the sun is several degrees warmer (about 5° to 7° C.) than the air.

2. Relative humidity may also affect the leaf temperature as compared to the surrounding air but to a lesser extent than the movement of the air about the leaf.

3. The sugar cane leaf in the shade is slightly cooler (about 1° C.) than the surrounding air.

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BRIEF PAPERS

SEASONAL VARIATION IN ELONGATION OF YOUNG SEEDLING RADICLES

In October, 1935, we made determinations of the elongation during a twenty-four hour period, at optimum temperature, of the young seedling radicles of lentil, cress, and sunflower. When we repeated the tests in January 1936, we found the elongation in each instance to be decidedly less. But another series of tests made in May 1936—using, of course, seed from the same lots—gave an elongation equal to or greater than that observed the previous October.

Methods and results

Seeds of lentil (*Lens esculentum* L.), of cress (*Lepidium sativum* L.) and of sunflower (*Helianthus annuus* L.) were germinated on strips of blotting paper disposed vertically along the inside wall of a metal moist-chamber and kept wet by capillarity downwards, according to the method previously described by one of us (**1**). When the young radicles were about 5 cm. long, the seedlings, still attached to the strips of blotting paper, were placed with the radicles suspended vertically along the inside wall of a moist chamber consisting of a 500-cc. pyrex beaker (tall form) covered with a petri dish, and this again placed in a 6 x 9-inch battery jar covered with a glass dish. Water on the bottom of beaker and battery jar, and wet blotting paper along the inside wall of each, insured the maintenance of atmospheric humidity close to saturation. Strips of paper over the upper edge of beaker and of battery jar kept the glass cover in each case slightly raised, and permitted circulation of air.

The moist chambers containing the seedlings were placed in a metal-lined incubator with adjustable temperature constant to $\pm \frac{1}{2}^\circ$ C. A fan within the incubator chamber promoted circulation of air and rapid adjustment of temperature. The test period was twenty-four hours. At the start of the

TABLE I

ELONGATION OF YOUNG SEEDLING RADICLES IN MOIST CHAMBER

PLANT	TEMP.	AVERAGE ELONGATION IN 24 HOURS		
		OCT. 1935	JAN. 1936	MAY 1936
	°C.	mm.	mm.	mm.
Lentil	28	27	19	34
Cress	28	33	20	40
Sunflower	32	66	35	60

test the paper strip bearing the seedling was in each instance cut at the length of the radicle; the extension of the radicle beyond the cut end of the strip at the end of the test period was, therefore, a measure of its elongation during that period. The figures in the following table represent, in each case, the average of not less than 30 measurements.

Discussion

HOAGLAND (**2**) has noted a marked seasonal variation in root growth of barley plants in solution culture, which he is inclined to ascribe to seasonal differences in quality or intensity of illumination. Likewise, WHITE (**3**) has observed a seasonal fluctuation in the growth rates of excised tomato root tips, which he considers explainable by variations in laboratory temperature. But our determinations of amount of elongation of radicles in a twenty-four hour period were made in a fairly light-tight incubator in which the set temperature was maintained to $\pm \frac{1}{2}^\circ$ C., so that the seasonal variations in amount of elongation noted by us cannot well be attributed to differences of illumination or of temperature during the test period.

Instead, we are inclined to associate them with the seasonal differences in ventilation conditions in the laboratory. Doors and windows are kept closed more during the winter months; and consequently, it is to be expected that during the cold weather season the air in the laboratory tends to contain higher concentrations of the vapor of compounds contained in, or associated with the use of illuminating gas, paints and oils, varnishes, lacquers, and the like. We have found a number of such substances, even when in extremely low concentration in the air, to have marked influence on the elongation of young seedling radicles.—M. A. RAINES and C. W. TRAVIS, *Howard University, Washington, D. C.*

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APPLICATION OF THE CHLORATE METHOD FOR DETERMINING NITROGEN TO LIGHT, FLUFFY MATERIALS[1]

In using the chlorate method[2] for determining total nitrogen in plant tissues, it was found that certain coarse, fluffy materials tend to float on the acid and to be carbonized, out of contact with the oxidizing reagent. This causes loss of nitrogen. The method, as previously described gives satisfactory results on green vegetative and woody tissue, since they are heavy and stay in the oxidizing solution. Further work on the method shows that fluffy materials, such as dry grass, must at all times be in contact with excess of oxidizing reagent during the heating period, or oxides of nitrogen are likely to be lost.

To make the method reliable for a wider range of materials and to make it unnecessary to grind very fine, the following modified procedure is recommended:

Put 100 to 300 mg. of dry tissue, or 500–1500 mg. of green tissue, into a 200-cc. Erlenmeyer flask. There should be at least 0.5 mg. of nitrogen in the sample used. Add 2 cc. of 50 per cent. sodium chlorate solution for each 100 mg. of dry sample, or each 500 mg. of green sample. Mix well with the sample and allow to soak a few seconds. Add 25 cc. of 52 per cent. by volume sulphuric acid and attach the flask to a water-cooled reflux condenser. Arrange the connections to the condenser in such a manner that the flask can be shaken readily. Heat rather slowly at first. When the reaction begins to become active, shake to wash down particles from the sides. Do not allow blackening of particles on the sides of the flask if it can be helped. However, if the particles of samples have been soaked with a 50 per cent. chlorate solution some blackening does no harm. Heat until the sample is completely gone (2–3 minutes). Stop the heating while there is still a very faint yellow chlorine color. Flush out the condenser with two successive 5-cc. portions of 52 per cent. H_2SO_4, and cool the flask in a cold water bath. After detaching, cool to room temperature.

Make the cooled solution up to a convenient volume. If the quantity of nitrogen present is small, the volume should be as small as possible. Immediately put exactly 1 or 2 cc. (use an accurately calibrated pipette) of the solution into a 25-cc. test tube and add 3 or 6 cc. of phenoldisulphonic acid, mix well, and allow to stand about ½ minute. (If a very reddish solution forms here, the heating was not continued long enough to decompose excess chloric acid. The solution should remain almost colorless.) Wash into a

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] EMMERT, E. M. Method for quickly determining nitrogen in plants, and soluble nitrogen as a measure of the nitrogen available for anabolic processes. Plant Physiol. 10: 355–364. 1935.

200-cc. Erlenmeyer with about 10 or 20 cc. of water and make alkaline with 40 per cent. NaOH solution, adding it until the maximum yellow color is produced and a small excess of alkali is present. Make to a volume which brings the yellow color near to the standard. After thorough mixing, the clear solution is compared with a standard (1 cc. = 0.0025 mg. N) in a colorimeter.

Discussion

Soaking with chlorate solution causes all particles to be completely oxidized without loss of oxides of nitrogen. Close checks with the Kjeldahl method were obtained with all types of samples tried so far, except pyridine and quinoline; but even the Kjeldahl method does not give accurate results with these compounds. Quite coarse samples are digested rapidly. A very important point is to cool the solution to room temperature after digestion and before adding the phenoldisulphonic acid. In hot solutions there is danger of loss of nitrosyl chloride, and even oxides of nitrogen, but there is no loss if the reaction between phenoldisulphonic and nitric acids takes place at room temperature. This point was not emphasized in the previous description of the method, and loss of nitrogen oxides may cause low results if precautions to cool to room temperature are not taken.

The following table presents results obtained on fluffy, coarse samples:

	Chlorate method	Kjeldahl method
1. Bluegrass	2.22	2.10
2. "	2.80	2.91
3. "	2.59	2.64
4. "	2.20	2.15
5. "	2.15	2.16
6. Oats	3.70	3.68
7. Alfalfa	3.81	3.74
8. Tobacco	5.03	5.05

Each of the determinations reported in the table was completed in less than ten minutes by the chlorate method, while the Kjeldahl method required 6 to 8 hours. Several Erlenmeyers, a reflux condenser, a pipette, test tubes, and a colorimeter are all the apparatus needed for the chlorate method; whereas the usual digestion shelves, distillation equipment, and burettes are needed for the Kjeldahl method. The reagents and standards needed for the chlorate method also are simpler to prepare.

If samples of plant extracts larger than 0.5 cc. are run for soluble nitrogen as previously described the precaution of cooling the solution before adding phenoldisulphonic acid must be exercised. On samples of 0.5 cc. or less, the shaking and blowing out of the chlorine usually cools the solu-

tion sufficiently; but it is best to allow the solution to cool even in smaller samples.—E. M. EMMERT, *Kentucky Agricultural Experiment Station, Lexington, Kentucky.*

CHLOROPHYLL IN SULTANINA GRAPES AND RAISINS

During ripening, the Sultanina or Thompson seedless grape changes from a green to an amber color. After treatment with sulphur dioxide and drying, the resulting raisin varies in color from amber with a greenish tint, to amber, and dark brown. In this paper is presented evidence identifying the green coloring matter with chlorophyll. The concentration of chlorophyll is so low, (see table I) that the figures given, particularly for the raisins, cannot represent much greater accuracy than that of the correct order of magnitude. The extracts of the sulphured raisins, furthermore, were markedly less stable than those of the fresh grape, and were decolorized in the course of a few hours of exposure to light.

The persistence of unaltered chlorophyll in the raisin may be explained on the assumption that it is inaccessible to the organic acids and to sulphur dioxide, which is known to be largely bound in organic complexes, presumably sulphonic acids. The pigment *in situ* might therefore be expected to survive intact. Extraction, whereby pigment and sulphur dioxide may be brought into the same phase, would lead to the ultimate destruction of the chlorophyll, particularly when exposed to light.

In the destruction of chlorophyll by light it is uncertain whether the process in its early stages is hydrolytic or oxidative or a combination of both, and the absence of well defined colored degradation products *in situ* (with one exception, phaeophytin) has rendered well nigh impossible studies of the reactions involved. Nor can much be said as to the effect of sulphur dioxide on chlorophyll. It is known that the primary effect of weak acids, including sulphur dioxide, is the loss of magnesium, with the formation of phaeophytin (**1, 3**). Subsequent loss of pigment may involve reactions having nothing to do with the sulphur dioxide.

In the grape and raisin extracts examined, no absorption band with a maximum at 5350 Å was observed, indicating the absence of phaeophytin. Experiments to be reported later indicate a definite reaction rate for the conversion of chlorophyll to phaeophytin, which the writer is measuring, in collaboration with M. A. JOSLYN.

The transmissions of the various chlorophyll solutions from grapes and raisins were measured spectrophotometrically at the position of maximum absorption for the characteristic red band. When compared with standard chlorophyll solutions, and with leaf extracts, it was found that the maximum was apparently shifted 60 Å toward the red. The solutions were then compared with a chlorophyll solution of such dilution that at their apparent

maxima, 60 Å apart, they had identical transmission values. When these solutions were inserted into the two light paths of the spectrophotometer, the displacement of the whole band was plainly visible, for both grape and raisin extracts, with respect to that of the standard. Less subjective methods indicate the shift of band is real, that of the maximum illusory, owing to material change in the skewness of absorption.

The most obvious explanation is that the ratio of the components, chlorophyll *a* to chlorophyll *b*, is markedly greater in these extracts than that found by WILLSTÄTTER and STOLL (**4**) for normal green leaves, where the ratio is somewhat less than 3 : 1. An examination of the individual absorption curves for the two components as given by WINTERSTEIN and STEIN, (5) and by ZSCHEILE, (**6**) shows clearly that the effect of increasing the ratio must be to translate the band further toward the red for a given photometer setting, and to increase the skewness. Additional references on the values of this ratio will be found in the work of WILLSTÄTTER and STOLL (*loc. cit.*), and of INMAN, (**2**).

Experimentation

Observations were made on two lots of grapes, those with a definitely greenish tint and those amber in color. The grapes, in lots of 1.2 kg., each were crushed, dehydrated with acetone, and extracted with the same solvent by steeping overnight. The acetone extracts were filtered on a Buchner funnel, the pulp being leached with fresh solvent. The acetone extracts (2 liters) were transferred by successive additions to a smaller quantity (200 cc.) of re-distilled hexane (b. p. 66–68° C.) each addition being followed by removal of the acetone with water. In this way, concentration of the solution was effected without recourse to heating. The solutions were dried over anhydrous sodium sulphate, decanted, and with rinsings made to volume (250 cc.).

Raisins were sorted into three groups: (a) greenish tint, (b) amber, (c) dark brown. The raisins, (0.7 kg.), were passed through a food chopper, and thoroughly mixed with acetone, as in the previous case. After extraction, steeping with fresh solvent yielded no further coloring matter. The acetone extract from the dark brown raisins yielded no pigment, and, therefore, was discarded.

The hexane solutions were in all cases yellow to greenish yellow. Absorption maxima, determined visually, were apparently at 6710 Å, and for chlorophyll (5 X, American Chlorophyll Company), and grass extract at 6650 Å $\pm$ 15 Å.

It was assumed that the absorption coefficients for the two would be equal at their apparent visually determined maxima. This introduces an error since the coefficient for pure *a* is greater than that for an approxi-

mately 3:1 mixture of $a:b$. In view of the low concentrations present, more precise measurements were not justified. Spectrophotometric comparisons were then made, with all instrumental factors kept constant. The chlorophyll concentrations were thus calculated, in mg./kg. actual and dry weight, assuming average values of 75 per cent. moisture for the grapes, 15 per cent. for the raisins. The results are given in table I.

TABLE I

CHLOROPHYLL IN SULTANINA GRAPES AND RAISINS IN MG./KG.

MATERIAL	ACTUAL WEIGHT	DRY WEIGHT
Grapes		
Greenish	1.2	5.0
Amber	0.5	2.0
Raisins		
Greenish	0.5	0.6
Amber	0.35	0.4

If we take as an average, for green leaves, a chlorophyll content of 8 gm. per kg. of dried leaves, it is seen that chlorophyll in mature grapes is less than 10^{-3} times that occurring in leaves.

Small quantities of other acetone extracts were transferred to ether and the following tests were made. The Molisch phase test was unsatisfactory for raisin extracts, presumably owing to the presence of sulphites, though a brownish phase appeared. The yellowish green color of the original solution made this of doubtful value in all cases.

No coloring matter was extracted from ethereal solutions of any of the samples with 22 per cent. hydrochloric acid.

N/100 alkali extracted the pigment from ether only in the case of sulphured extracts not freshly prepared, indicating the formation of free carboxyl groups.

Summary

Extremely low concentrations of chlorophyll have been found in Sultanina grapes and raisins. The evidence indicates that the ratio of the two components, *a* and *b*, is greater than that of normal green leaves, and that the chlorophyll is substantially unaltered *in situ*, but decomposes rather rapidly, on extraction, if the original has been treated with sulphur dioxide. —G. MACKINNEY, *University of California.*

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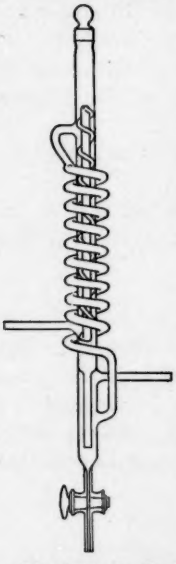
THE QUANTITY OF ETHYLENE PRESENT IN APPLES[1]

(WITH ONE FIGURE)

A rapid chemical method for the determination of ethylene in plant tissues has been devised. Its main details follow: 250 gm. of tissue are placed in a 500-ml. flask together with 100 ml. of water. A reflux condenser, to the upper end of which is attached an evacuated gas sampling tube of 330-ml. capacity, is connected to the flask by means of a ground glass joint. Connection is established between the tube and the flask, and the water and tissue in the flask are boiled until the tissue has broken down, and all the gases present in the flask and tissue have been driven into the gas sampling tube. The tube is then detached, and the gases contained therein are passed over sodamide to remove interfering substances such as alcohol, acetaldehyde, and esters, and finally through 5 ml. of 0.01 N postassium permanganate solution in an absorption unit of special design (fig. 1) which permits 5 minutes contact with the absorbent when the gas is passed through at the rate of 5 cc. per minute. Ethylene is estimated by pipetting an aliquot of the absorbent into excess ferrous sulphate, and titrating the unoxidized ferrous ion with 0.002 N permanganate. Under the conditions of the procedure, the reduction of permanganate is 8 per cent. over the value calculated for the oxidation of ethylene to ethylene glycol.



[1] Published with the permission of the Director of the Minnesota Agricultural Experiment Station as Scientific Journal Series Paper no. 1436.

Using this procedure, determinations of the ethylene content of MacIntosh apples after nine months of storage have been made. These showed an ethylene content of the order of 0.12 mg. per kilogram of fresh tissue.

Calculations from the data of DENNY and MILLER[2] show that they estimated, by the study of epinastic responses induced by apple tissue, an ethylene content of about 0.2 mg. per kilogram of apple tissue, which is in fair agreement with the present results.

It is interesting to note that, expressed on a volume basis, this figure represents an ethylene content of about 1:10,000 in the tissue, which is equal to the concentration calculated to be present in fruits ripened artificially in a chamber in which the ethylene in air concentration has been adjusted to 1:1000, the concentration commonly used in this practice.

Further research on the relation between ethylene content and keeping quality of apples is being conducted at this laboratory.—R. C. NELSON, *Minnesota Agricultural Experiment Station.*

A TEACHERS' FORUM

At the Kingston meeting of the New England section, the closing session was devoted to a seminar on the teaching of plant physiology. About 40 teachers followed closely a lively discussion of the subject: "What are the logical objectives and methods of approach in teaching plant physiology?". The group found the teachers' problems so important and complex as to warrant further consideration of them at future meetings of the section.

This seminar on teaching methods was an innovation, designed to balance the program of an annual meeting with the interests of the society membership. At the request of the local committee for the Rhode Island meeting, it was organized and conducted by a committee of teachers with Dr. C. J. LYON of Dartmouth as chairman. The basic thought was to make the instruction in elementary physiology more effective and of more real value to college students.

The forum was opened with statements of views prepared by teachers in the two types of institutions, liberal arts colleges, and state universities. Dr. GEORGE P. STEINBAUER of Maine showed how students in his courses came prepared in botany and chemistry and in search of detailed information bearing on their future work in such fields as forestry and horticulture. The written statement of Dr. H. E. PULLING of Wellesley, read by Dr. W. REI ROBBINS of New Jersey, emphasized the necessity in liberal arts colleges of teaching plant physiology as a necessary, important part of biology, and as a basis for understanding all life in relation to its environment.

[2] DENNY, F. E., and MILLER, LAWRENCE P. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. Contrib. Boyce Thompson Inst. 7: 97–102. 1935.

The program continued with a demonstration of teaching a topic, by two different methods—the plain lecture full of textbook facts, *versus* demonstration interspersed with questions and references to the personal experiences of the class members. Dr. LINUS H. JONES of Massachusetts State College and Dr. DOROTHY DAY of Smith cooperated in this difficult assignment and with great success.

The open discussion which followed centered on these contrasting classroom methods and their relation to laboratory instruction, prerequisites to physiology courses, teacher personality, and the general objectives of courses. There was a general agreement on the principle of using the lecture-demonstration method for qualitative work, combined, if possible, with quantitative studies in the laboratory. The relative unimportance of specific method, in contrast with the ability and personal qualities of the teacher, was left as an unchallenged observation. The question of requiring training in organic or physical chemistry previous to the course in plant physiology brought out the great differences in the status of physiology courses in universities and liberal arts colleges. Though admittedly desirable for teaching the basic facts of the science, a chemistry requirement is probably impossible in institutions which train primarily for society, or for non-scientific professions. The other questions and answers emphasized the need for each teacher to work out the method and procedure best suited to the individual school and instructor.

Within the time available for a single session, the group could not consider the details of teaching even a single phase of physiology along with the general features. In the words of one teacher present: "It was generally believed that this type of discussion should pave the way for consideration of specific material in the general course in plant physiology."

The exact subject to be taken up next year will be determined by the needs of the members but it is probable that instruction in such new or difficult topics as auxins, translocation, or osmotic relations will be chosen. The undersigned has been requested to organize the teachers' interests within the New England area and will welcome suggestion and other aid in carrying out the assignment.—CHARLES J. LYON, *Dartmouth College, Hanover, N. H.*

NOTES

Indianapolis Meeting.—The fourteenth annual meeting of the American Society of Plant Physiologists will be held at Indianapolis on December 28–30, 1937. The Hotel Claypool has been designated as headquarters of the society, as well as its meeting place, a most convenient arrangement.

As usual, the annual dinner will be held on the evening of the first day of the meeting, December 28. For many years the annual dinner has served as the time and place of announcements of awards of honors, election of corresponding members, the HALES addresses, memorials, etc. It has become the most important and interesting phase of our social contacts. Members and friends are reminded to obtain tickets promptly on arrival, at the time of registration with A.A.A.S. The dinner has always been open to all who cared to attend and enjoy the festivities.

Symposia and joint meetings have been arranged, and there will be the customary sessions for reading brief papers. On Thursday evening, there may be a session devoted to the problems of successful teaching of plant physiology. The success of the New England Section with such a meeting encourages the hope that there may be equal interest in a discussion of methods of presenting our material to students, and that a round-table conference following the discussion of the problems may aid in the improvement of our methods. This session for teaching problems will close the fourteenth annual meeting.

Every member in the middle west will be expected at the meetings, and as many as can come from more distant points. Especially it is hoped that all members of the executive committee may be present to consider important items of business and policy.

Early Payment of Dues.—The importance of paying dues early may not be apparent to all of our members. It has been customary to discontinue all memberships at the close of the calendar year, until they are renewed by payment of dues, to reduce losses from sending out copies of PLANT PHYSIOLOGY to those who do not intend to renew their memberships. The loss of copies of even one number of the official journal breaks volumes that are held in reserve for future members and subscribers. They are equivalent to cash assets. Moreover, when dues are paid between October 1 and December 31, the secretary-treasurer can retain the addressograph labels without danger of errors, and he can avoid the costs of replacement of labels which follow the discarding of them owing to late payments. Early payment also helps the editors to gauge their use of manuscripts, for they can then know early about what the probable income of the society will be for the ensuing journal

year. Cooperation of members and subscribers in this effort to avoid losses, errors, and additional costs will be greatly appreciated.

Charles Reid Barnes Life Membership Committee.—The committee appointed by the president of the society to select the recipient of the thirteenth award of the CHARLES REID BARNES life membership, in the American Society of Plant Physiologists is as follows: Dr. H. R. KRAYBILL, Purdue University, chairman; Dr. ELBERT T. BARTHOLOMEW, University of California; Dr. BERNARD S. MEYER, Ohio State University; Dr. FRANK B. WANN, Utah Agricultural Experiment Station; and Dr. FELIX G. GUSTAFSON, University of Michigan.

Program Committee.—The program committee always bears a heavy responsibility in connection with the meetings of the society. For the Indianapolis meeting, the committee is composed of the following members: Prof. R. B. WITHROW, chairman, Dr. J. D. SAYRE, and Dr. W. REI ROBBINS. Everything that can be done for the comfort, convenience, and entertainment of those who attend the annual meeting is being done with efficiency and dispatch. This loyal and faithful service is greatly appreciated by all concerned.

Executive Committee.—The membership of the society is entitled to know who constitute the personnel of the executive committee. There are twelve members, five *ex officio*, three elected, and four representatives of the organized sections. The full membership is as follows: Dr. OTIS F. CURTIS, chairman; Dr. R. B. HARVEY, Dr. WALTER F. LOEHWING, Dr. F. P. CULLINAN, Dr. C. A. SHULL, Dr. D. R. HOAGLAND, Dr. H. R. KRAYBILL, Dr. B. E. LIVINGSTON, Dr. B. E. GILBERT, Dr. D. M. DOTY, Dr. H. F. CLEMENTS, and Dr. R. W. LORENZ.

Chemical Methods.—A number of workers have found the first supplementary report of the Chemical Methods committee a satisfactory substitute for the original recommendations. As the original report is no longer available, the supplementary report is the only one that can be supplied. Many of our members have come into the society since these reports were published. The first supplementary report, with cover, costs only 15 cents, postpaid, or may be purchased in lots of eight for $1.00, if as many as that can be used. Those who may care to purchase a copy of the recommendations are advised to write to Dr. W. E. TOTTINGHAM, chairman of the committee, Agricultural Chemistry Building, University of Wisconsin, Madison.

Errata.—The customary list of errata found in the first three issues of volume 12 of PLANT PHYSIOLOGY is presented at the close of the table of con-

tents. If these errors are recorded in your copies of the journal you will be protected against the use of erroneous information at some future time. Assistance of all authors is requested to make these lists as complete as possible.

Vladimir Nikolaevitch Lubimenko.—The joy anticipated in presenting the portrait of the great Russian plant physiologist, LUBIMENKO, as a memento at or near his sixty-fifth birthday has been turned to sorrow by his death from a heart attack on September 14, 1937. He had proof-read and returned the short paper which opens this number of PLANT PHYSIOLOGY, but could not live to see it in published form. Dr. LUBIMENKO was a corresponding member of the American Society of Plant Physiologists, having been honored by election at the Pittsburgh meeting in 1934.

A few of the significant facts concerning his life and activities are presented here. Born on January 18, 1873, in the government of Voronej, on the frontier of Great Russia and the Ukraine, he grew up and was given his first education among his home surroundings. Formal education began as a teen-age boy at the School of Agriculture at Kharkov, which he attended from 1886 to 1892. At the age of 21 he entered the High School of Forestry at St. Petersburg, where he received a gold medal for his first work on plant anatomy. The interest aroused in these early years led him to specialize in botany, and he was assistant to the professor of botany, Dr. I. BORODIN, while yet a student in the High School of Forestry. He took up additional work at the University of St. Petersburg, where he obtained his diploma in 1902.

Following this work he enjoyed work in foreign lands. In 1903 his institution (High School of Forestry) sent him to Bonn, where he worked on cytology under STRASBURGER, and early the following year he went to the Sorbonne at Paris, for some work with BONNIER. In 1905 he obtained a commission from the Department of Forestry to study the influence of light on trees; he continued on at Paris, engaged in this work for three years.

Returning to Russia in 1908, he entered the service of the Botanical Gardens of Nikita in the Crimea, where he held the position of botanist for five years. He was awarded a master's degree in 1910 for his studies of photosynthesis, and in 1917 his doctor's degree for a major contribution on plastid pigments. In 1913 he again left his native land for foreign study and travel. He undertook a scientific journey to Australia and the Malay Islands, working a part of the time at the Buitenzorg Laboratory in Java. After this journey he began his long service for the Botanical Gardens at St. Petersburg, where he spent the remainder of his life. Invited there as botanist of the Museum, he was soon named director of the laboratory of plant physiology, which was created by him. When, after the revolution, a department of experimental botany was founded, LUBIMENKO became its director.

He served as special lecturer in various institutions, in St. Petersburg at the High School of Lesheft, 1916–1928; at the High School of Geography, later transformed into a Geographical Institute, 1916–1925; at the Chemical-Pharmaceutical Institute, 1922–1930; and at the Military Academy, 1924–1930.

Many honors came to him in recognition of his distinguished services. In 1913 his studies on chlorophyll in collaboration with MONTVERDE were recognized by the award of a prize by the Academy of Science, and again in 1918 the Academy of Science awarded him a prize for his studies on the transformation of the pigments of plastids in the living tissues of plants. It was at this juncture that he was called to the Scientific Institute of Leshaft where he continued his work to the end. In 1922 the Academy of Sciences U.S.S.R elected him a corresponding member. He was a delgate of the U.S.S.R at the International Congress of Protection of Nature Monuments at Paris in 1923, and during the summer of that year gave a series of lectures on plant physiology at the University of Paris. In 1925, having been sent again to France, he studied the physiology of Mediterranean algae at the Biological Station of Banijuls sur Mer. He was a delegate from the Academy of St. Petersburg to the fourth International Botanical Congress at Ithaca in 1926, and took an active part in the work of the Congress at the request of the Committee of Organization. He contributed a valuable paper on chlorophyll and the genesis of the photosynthetic apparatus of plants. In 1927 he became a member of the American Society of Plant Physiologists, in 1928 a member of the American Genetic Society, in 1935 a member of the oldest botanical society in France, La Societé Linnéenne de Lyon. The Academy of Science of Ukraine elected him as a member in 1929, and since that time he had served as director of its Department of Chemical Plant Physiology. In Russia he had been for many years a member of the Society of Naturalists and the Botanical Society.

His career as a scientific contributor began in 1900, and he was the author, either alone or jointly, of scores of papers which have enriched our knowledge of the physiology of plants. The end of his rich and fruitful life came swiftly, and apparently without warning, on September 14; for up to an hour before his death, he was writing at his writing-table.

In her bereavement we extend to his widow, INNA, our deepest sympathy. The high regard and esteem which her husband had attained personally and professionally in all lands, will be a precious memory to her.

George Perkins Clinton.—In the death of Dr. GEORGE PERKINS CLINTON, botanist of the Connecticut Agricultural Experiment Station, on August 13, 1937, the American Society of Plant Physiologists has lost one of its distinguished members. Although primarily a mycologist and pathologist, Dr. CLINTON was keenly interested in related botanical fields, and in botany as

a whole. He was born at Polo, Illinois, May 7, 1867, and had recently celebrated his seventieth birthday anniversary. His interest in agricultural pursuits and the pleasures of botanical collecting may have been stimulated by his early environment. His father was a rural journalist who had won honors from the College of Agriculture at the University of Illinois for his service to rural interests. As a boy GEORGE grew up in the atmosphere of a rural newspaper plant, and in a home where love of rural life was predominant. It was during this period that he began collecting herbarium specimens, a hobby that continued as a serious but most pleasant part of his vocation, throughout life.

His early collegiate training was received at the University of Illinois, at which institution he was awarded his B.S. degree in 1890, and M.S. degree in 1894. Later he entered Harvard, from which he received the M.S. degree in 1901, and Sc.D. in 1902. During this twelve-year period of training, he was assistant botanist at Illinois, and in the Illinois Agricultural Experiment Station. In 1902 he was called as botanist to the Connecticut Agricultural Experiment Station. Here he spent 35 years in mycological and pathological research, with considerable attention to the Ustilagineae, and accomplished much in the warfare against harmful fungal parasites of crop plants and forest trees. He also served as lecturer in forest pathology at Yale University from 1915 to 1926, and as research associate in botany at Yale from 1926 to 1929.

Among his important services may be mentioned his work in Porto Rico, where he was sent in 1904 to study coffee rust; his work at Harvard in 1908 on brown tail moth control; his expedition to Japan in 1909 to obtain parasites for control of the gypsy moth; his work on tobacco wild fire; and his travels for collection of fungi in Canada, Europe, Hawaii, Japan, Porto Rico, and Panama. His valuable collections of specimens, and his library of reprints were presented to the Experiment Station. The departmental library, and these fine collections of *exsiccati* constitute a monument to his foresight and wisdom in the management of his department.

He was a member of the New England Botanical Club, the Botanical Society of America, and many other scientific organizations. He had been honored as vice-president of Section G, A.A.A.S., in 1915; as president of the Phytopathological Society in 1912; as a fellow of the American Academy of Arts and Sciences; and as a member of the National Academy of Sciences.

He is survived by his wife, who lives in New Haven. Their only child, a son, was killed in France. All members of the American Society of Plant Physiologists join in extending heartfelt sympathy to Mrs. CLINTON in her loss.

Biochemistry of Cellulose, the Polyuronides, Lignin, etc.—A monograph of much more than usual interest and value is this volume by A. G.

NORMAN, biochemist at the Rothamsted Experimental Station. The development in recent years of new methods of studying the fundamental units of cellulose and other polysaccharides has brought about a much better understanding of the wall materials of plant cells than was possible even a few years ago.

There are eight sections to the volume, dealing with the following subjects: cellulose; polyuronide hemicelluloses; pentosans, hexosans, and hexopentosans; pectin; gums, mucilages, and gel-forming substances; lignin; metabolism of plant cell-wall constituents; and microbial polysaccharides. An appendix is devoted to the discussion of uronic acids and pentoses.

The value of this work is the insight which it permits regarding the biochemical processes involved in the formation of these materials during metabolism. In this sense it is a valuable contribution to true biochemistry, and not a mere discussion of the properties of biochemically produced plant products. The book is highly commended to all students of chemical plant physiology. Dr. NORMAN has presented his material with great skill.

The book contains 232 pages, 12 illustrations, author and subject indexes. It is published by the Oxford University Press at $5.00 per copy. Those desiring to place orders for it may address the Oxford University Press, 114 Fifth Ave., New York.

Phytohormones.—A new volume in the *Experimental Biology Monographs* series of the Macmillan Co. is an extremely interesting summary of the work thus far accomplished in the study of plant hormones. The authors are Dr. F. W. WENT and Dr. KENNETH V. THIMANN.

The introductory chapter outlines the task of the authors, defines the hormone as a substance which, formed in one part of an organism, is transported to some other part, where it influences a specific physiological process. Earlier reviews are mentioned briefly.

The succeeding chapters deal with the development of the hormone concept; the technique of auxin determinations; formation and occurrence of auxins; the relation between auxin and growth; auxin transport and polarity; chemistry of the auxins; mechanism of the action; growth of roots; tropisms; root formation; bud inhibitions; other activities of auxins; and general conclusions.

The authors have summarized the phenomena of hormone action very succinctly, for with a lengthy bibliography, and with subject and author indexes, the book runs to only 294 pages. Although it appears only a year later than the BOYSEN-JENSEN translation by AVERY and BURKHOLDER, the authors feel that this new monograph aims more at an analysis of the problems, and the integration of the accumulated material, than has any previous summary. It is difficult to do this adequately with so many new contributions coming out each year, and with the controversial points not all settled.

The final chapters will be very helpful to students who wish to grasp quickly the general implications of the vast plant hormone literature now on record.

Every student of plant physiology should have a copy of this work, which is a timely and valuable digest of this field of research. The book is quoted at $4.00 per copy by the publishers. Orders may be sent to the Macmillan Co., 60 Fifth Ave., New York.

Potash Deficiency Symptoms.—Through the cooperation of the German, French, and American potash industries, a valuable and unique summary of potash deficiency symptoms has been prepared by OSCAR ECKSTEIN, ALBERT BRUNO, and J. W. TURRENTINE, with collaboration by G. A. COWIE and G. N. HOFFER. The book is profusely illustrated, with 41 text figures, and 54 beautiful colored plates depicting the deficiency symptoms in natural colors. There is also a colored chart showing the amounts of plant foods removed from the soil by agricultural and horticultural crop plants. Another feature of the work that will meet universal approval is the fact that the information, including the index, is printed in three languages, German, French, and English.

Following the introduction, the first part of the book deals with general symptoms of potash deficiency. This includes the effects on the leaf, root system, blossom and fruit, changes in external appearance, and modifications of the inner structures; the secondary effects of potash deficiency, especially the lowered resistance to diseases, insect pests, and untoward climatic conditions; the market value of crops in relation to potash deficiency; and the pathology of potash deficiency.

The second part deals with the specific effects on various cultivated crops. HOFFER contributes the section on maize and other cereals, and COWIE the section on fruit trees. A brief reference to deficiency symptoms of grape vines concludes the text. The 54 colored plates illustrate these specific responses, and are accompanied by brief descriptions of the symptoms shown by each crop plant. The bibliography consists of 209 titles, and there is an adequate index. The price is remarkably modest, only $2.25 per copy. It should find a welcome place in every plant physiologist's private library. Distribution is through B. Westermann and Co., Inc., 24 West 48th St., New York.

Pathology of the Plant Cell.—The second volume of ERNST KÜSTER'S *Pathologie der Pflanzenzelle* has appeared as volume 13 of the *Protoplasma Monographien*, published by Gebrüder Borntraeger, W 35 Koester Ufer 17, Berlin. This work considers particularly the pathology of the plastids. The two chapters deal with modifications in form, and modifications in structure, of the various plastids of plant cells. Mechanical form modifications, abnormal growth forms, deformities from capillary contraction and expansion,

abnormal divisions, and reductions in size by loss of substance are detailed in the first chapter. The second chapter takes up important structural modifications of the plastids in connection with starch storage and the presence of active pyrenoids; agglutination phenomena; swelling and vacuolization; lipophanerose (fatty degeneration); water loss; and necrotic changes. An appendix considers pigment breakdown, and the division of the chloroplasts. More than 200 titles are cited in the bibliography; and subject and author indexes conclude the work. It is a valuable and worthy companion to KÜSTER's original monograph in the same series published about eight years ago. The price of the book bound in red cloth is RM 16. The publishers will give prompt attention to orders placed with them.

Mitogenetic Radiation.—Under the auspices of the Committee on Radiation of the National Research Council, ALEXANDER HOLLAENDER and WALTER D. CLAUS have made an experimental study of the problem of mitogenetic radiation. Their report is embodied in Bulletin no. 100 of the National Research Council. The work was done at the University of Wisconsin under the supervision of Dr. B. M. DUGGAR, which is a sufficient guarantee that the work was carefully planned and executed.

The results are negative. No such radiations have been detected in connection with the activity of typical biological "mitogenetic senders." The work of GURWITSCH and his followers in Europe remains entirely unconfirmed. One cannot accept the idea that these typical mitogenetic ray sources, grown in Europe, possess the property of radiation, and grown in America lack this radiation capacity. It would also be very difficult to understand how so many people could be led astray in European laboratories if mitogenetic radiations prove to be nothing but a myth. While the authors do not admit that their report is necessarily the "swan song" of mitogenetic research in the United States, it may turn out to be just that. There would seem small excuse for additional attempts to find them unless unequivocal evidence can be supplied that the methods here used are at fault, or that the investigators have not had the requisite skill to detect the radiations. The reviewer doubts that such evidence will be forthcoming.

This Bulletin no. 100 may be purchased from the National Research Council at $1.00 per copy.

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[1] Papers published in PLANT PHYSIOLOGY are indexed in *The Agricultural Index*, The H. W. Wilson Co., New York.

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